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Hesperocyparis abramsiana
var. *butanoensis*



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**CHROMISTA REVISITED: A DILEMMA OF OVERLAPPING
PUTATIVE KINGDOMS, AND THE ATTEMPTED
APPLICATION OF THE BOTANICAL CODE OF
NOMENCLATURE**

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ABSTRACT

It was many centuries before it was realized that all organisms were not either plants or animals, and many more years before it was understood that the catch-all kingdom "Protista"—proposed to include predominantly unicellular, "non-plants" and "non-animals"—was heterogeneous and phylogenetically inadequate, encompassing both related and unrelated organisms. The probable unity of a particular group of protists—viewed as chromophytous algae or pseudofungi (and related protozoal forms), and often exhibiting a characteristic heterodynamic flagellar pattern—was gradually understood; these became separated from Protista, and recognized by various kingdom, subkingdom, phylum, or subphylum names. At kingdom level, two names and groupings—Chromista and Stramenopiles—have competed, among others, for these typically heterokont protists, in a partial overlay of descriptive information. Stramenopiles have the "tighter" circumscription, by virtue of *definition* based on the occurrence of unique, composite, tubular, flagellar hairs. Acceptance of Stramenopiles (as a more clearly monophyletic group) was beginning to hold sway over the more diverse Chromista (with its less obviously related major sub-groupings). However, recent evidence from plastid evolution has suggested that the larger, yet still generally monophyletic, assemblage of (mostly) heterokont protists—the Chromista—remains viable as a putative kingdom, much in the original sense of Cavalier-Smith (1981, 1986). Although the matter is still equivocal, the present paper notes a return to usage of the kingdom name Chromista—representing an assemblage including not only stramenopilous organisms, but also plastid-related groups, i.e., haptophytes and cryptomonads. The naming of chromistans has fallen by tradition mainly under the botanical code of nomenclature, which, as the other

major eukaryotic code (the zoological code), contains little regulation at kingdom level. Difficulty in properly establishing kingdoms, such as Chromista, or Straminipila, might be alleviated if a unified code of (biological) nomenclature were developed, with guidelines for determining/composing kingdom names. As a further point of present code deficiency, supra-kingdom ranks (to which yet larger groupings such as "Chromalveolata" might be assigned) are not recognized in existing, formal codes (botanical, zoological, or bacteriological)—a situation that could also be changed through code unification. It is important to examine current, proposed, *ad hoc* naming schemes in context of present nomenclatural codes (one of the points of this paper). It would be gratifying if systematists that produce future encompassing taxonomies (major eukaryotic schemata), and those involved with the development of a future code (or codes) of nomenclature, could work in consort toward the goal of improved, stable systems of classification—systems not only modern and biologically accurate, but nomenclaturally appurtenant as well. *Phytologia* 91(2): 191-225 (August, 2009).

KEY WORDS: Chromalveolata, Chromista, codes of nomenclature, code unification, complex plastids, Domain, endosymbiosis, heterodynamic flagella, Heterokonta, holophyletic, Kingdom, monophyletic, Ochrista, Pseudofungi, Stramenopila, Straminipila, supergroup, Supra-kingdom, tubular mastigonemes, tubular mitochondrial cristae.

Botanical nomenclatural regulation is lax at ranks above Family, especially for names not automatically typified—consider Articles 16 and 17, *International Code of Botanical Nomenclature* (ICBN), McNeill et al. (2006). Nomenclature at these "higher" levels can be confusing. At the rank of Class, three different terminations for names are suggested (Recommendation 16A.3, ICBN), depending on the "kind" of "plant" in question. This can result in rather closely related plant groups—e.g., liverworts, compared with their probable relatives, the charophytes (cf. Niklas, 1997; Blackwell, 2003)—having quite different sounding Class names. At Division/Phylum level, although name-terminations are reduced to two (i.e., for "plants" vs. "fungi"), the dual usage in botany now—i.e., Division *or* Phylum (Recommendation 16A.1)—is in itself confusing. At Kingdom level,

there are essentially no *Code* instructions on how to name these major groups. There are, for example, no guidelines for kingdom name terminations. By convention, such names often end in “a”—e.g., Animalia, Archaeobacteria, Biliphyta, Monera—but this is not uniformly followed, e.g., Fungi, Plantae, Viridiplantae. Jeffrey (1971) proposed that the suffix “-biota,” implying neither plant, nor animal, nor fungus (nor bacterium, for that matter), be used as a standard, “neutral” ending for kingdom names; however, this proposal was not widely adopted.

There is, in fact, a paucity of nomenclatural regulation by the botanical code and (particularly) by the zoological code of higher categories such as Phylum (Division), Class, and even Order. In the case of the botanical code, such “weak regulation” has been attributed to the belief that these “upper groups” are too unstable or uncertain in delimitation (cf. Gledhill, 1989) for application of rigorous nomenclature—such as the principle of priority, or the type method (with the exception of automatically typified names, cf. Article 16.2, ICBN, 2006; but even this does not clearly apply to Kingdom). The dearth of rules and recommendations at the level of Kingdom, however, is perhaps more of an historical artifact. Until the mid-nineteenth century, it was believed that there were just plant and animal kingdoms—hence, no need for detailed regulation of names of kingdoms (because the matter was non-controversial). This changed, though, when authors such as Hogg (1860) and Haeckel (1866) recognized, additionally, a “protocist” (or “protist”) kingdom for (mostly) unicellular organisms, not clearly plant or animal. While some later authors (e.g., Copeland, 1956; Whittaker, 1959) continued to emphasize (and increase the recognition of) kingdoms, others (e.g., Christensen, 1958) came to believe that Kingdom was a relatively meaningless, perhaps artificial, grouping category, and recommended removal of Regnum (Kingdom) from the botanical code. Christensen’s proposal (1958) did not gain favor, however, and the ICBN (2006) still recognizes “Kingdom” (Article 3.1)—even though specific rules for “regulating” this category remain absent.

By mid-twentieth century, the number of kingdoms generally accepted had risen to five (cf. Whittaker, 1969). More recently, six to nine kingdoms have often been recognized (cf. Edwards, 1976; Cavalier-Smith, 1981, 1987, 1993, 2004; Corliss, 1994; Blackwell and

Powell, 1995, 1999; Blackwell, 2004). With proliferation of kingdoms came the additional complication of the supra-kingdom category, "Domain" (Woese et al., 1990)—a rank not sanctioned by codes of nomenclature. In any case, more kingdoms or kingdom-level groups are presently recognized than there are codes of nomenclature (There are three major organism-based codes, discussed below). Several different kingdoms, or parts of kingdoms, are under the umbrella of the botanical code alone (cf. Blackwell and Powell, 1999; Blackwell, 2008)—e.g., Fungi, Myxomycetes (these being Protozoa, cf. Corliss, 1991), Cyanobacteria, and most Stramenopiles (cf. Blackwell and Powell, 1999)—in addition to plants, obviously the intended objects of this code. It may be controversial which code should control the nomenclature of a given group of organisms, e.g., Cyanobacteria—in this case, the botanical or the bacteriological code (cf. Blackwell, 2008).

So, questions remain: Were the kingdoms that we now recognize properly established, based on both biology and nomenclature? If not, how should they be established? Is it possible to determine if a given kingdom name is technically accurate and properly applied? Is, for example, the kingdom name-termination appropriate?—On what basis is this decided? And, under which code of nomenclature should each putative kingdom be "governed?" Cavalier-Smith (1978) and Corliss (1983) initially raised questions concerning possible nomenclatural consequences of creating multiple kingdoms of organisms, a situation readdressed by Blackwell and Powell (1999), and that pertains here. These sorts of questions do not necessarily have ready answers, nor will I seek to deal with all such questions here (and certainly not for all kingdoms). What I wish to address is a special confusion concerning two proposed kingdoms—Chromista and Stramenopila—that have similar, yet clearly non-identical, circumscriptions; i.e., they are descriptively over-lapping. I give, subsequently, particular consideration to determination of the usage and best application of these particular names and groupings, and to the various complications that are attendant.

CHROMISTANS, STRAMENOPILES, AND THE BOTANICAL CODE

The case in point here, Chromista vs. Stramenopiles, is which kingdom and name to recognize? In addressing this question, it should be decided under which code the nomenclature of these groups should fall. Organisms recognized variously as chromistans and stramenopiles are neither plants nor animals (cf. Patterson, 1989; Cavalier-Smith, 1987; Keeling, 2004). Historically, however, the naming of the majority of the membership of either alleged kingdom has been in accordance with the botanical code (cf. Blackwell and Powell, 1999); thus, this question is decided (for now), by precedent, in favor of the ICBN. Although this decision is perhaps more clear-cut with Stramenopiles, it is nonetheless true that, since the Chromista are also largely characterized by chromophytous algal (e.g., chrysophytes, xanthophytes, phaeophytes, diatoms) and by pseudofungal lineages (e.g., Oomycetes), cf. Cavalier-Smith (1989), their nomenclature generally forfeits to the botanical code as well (further discussed below). To a lesser extent, some members of "both" putative kingdom groups have been named under the zoological code (e.g., certain amoeboid or colonial chrysophytes).

We are saddled at present with the situation of having two separate eukaryotic codes, botanical and zoological, plus the bacteriological or prokaryotic code—hence, three major organismal codes (and, in addition, a code for viruses, and a specialty code for cultivated plants). In hindsight, it is apparent that none of these codes is a good fit (in biological context) for the naming of organisms considered herein (chromistans/stramenopiles). Furthermore, since the three main codes were each conceived (more or less independently) to facilitate nomenclature of members of the plant, animal and bacterial kingdoms respectively, the nomenclature in each code (with some exception in the bacteriological code) effectively starts *below* the level of kingdom. The naming procedure for the category of kingdom is substantially neglected in the botanical code, and even more so in the zoological code. If, however, there were a unified code (cf. Cavalier-Smith, 1978; Patterson, 1986; Corliss, 1990; Blackwell and Powell, 1999; Blackwell, 2008), the question of which code should cover which kingdom (e.g., Chromista) need not be asked, since nomenclature of all

organisms (regardless of their “biology”) would be under one code, and rules would doubtless be in place for kingdom names.

But since there is as yet no accepted unified code, the nomenclatural default in the case of all eukaryotes (regardless of relationships) is, at present, to either the botanical code or the zoological code. Whereas some organisms considered to be Chromistans (and Stramenopiles), such as the more protozoal representatives (e.g., the primitive Bicoecids), have often fallen under the zoological code, the majority are (as indicated) still under the governance of the botanical code—since most chromistal/stramenopilous organisms, accurately or not, have typically been referred to as either “algae” or “fungi” (“groupings” traditionally covered by the botanical code). In the case of “fungi,” however, it should be noted that none of the “chromistal fungi” are actual Fungi, but rather are “Pseudofungi”—e.g., Oomycetes, Hyphochytriomycetes, and the more protozoan-like Labyrinthulids—these pseudofungi being relatively unrelated to true Fungi (Cavalier-Smith, 1986, 1989; Alexopoulos et al., 1996; Blackwell and Powell, 2000) and more closely related to types of (alveolate) Protozoa, such as ciliates, dinoflagellates, and apicomplexans (cf. Cavalier-Smith et al., 1995; Van de Peer and De Wachter, 1997; Keeling, 2004). Nonetheless, pseudofungi continue to be “covered” (as do true fungi) by the botanical code—evidenced, for example, by the reference to Oomycetes in item number 7 of the Preamble (ICBN, 2006). “Algae,” no longer considered a cohesive phylogenetic construct (cf. Van den Hoek et al., 1995, p. 9), are still also generally treated, operationally, as “plants” by the ICBN. It remains equivocal whether the nomenclature of certain groups of organisms (in the Chromista) such as the Pedinellids (cf. Patterson, 1989) and Silicoflagellates (cf. Tappan, 1980)—difficult to pigeonhole as “algae” vs. “protozoa”—should be considered, presently, under the botanical or the zoological code.

In opting at the present time primarily for the botanical code, based on overall membership of assemblages considered here, it might be assumed that we would thereby know what rules to follow in establishing the appropriate kingdom (Chromista or Stramenopila, or other competing names/groupings subsequently discussed). But since there essentially aren’t any “kingdom rules” per se—being as which

name came first is largely irrelevant (priority is not binding at kingdom level, as extrapolated by comparing Principle IV with Article 11, ICBN)—and since none of these names, arguably, is really a typified name (see later discussion, however, concerning Chromista)—such decisions boil down more to a matter of informed preference than code “legality.” This preference is informed, mainly, by asking biologically based questions, such as: Which grouping is the most monophyletic (pertinent, if one is striving for phylogenetic nomenclature, cf. de Queiroz and Gauthier, 1992; de Queiroz, 1997, 2006; Cantino, 2000)? Even though a *PhyloCode* has not been formally endorsed by any official, international nomenclatural congress (other than, perhaps, that of those promoting the *PhyloCode*, cf. de Queiroz, 2006), it would nonetheless seem logical that at kingdom level we would wish to recognize a group that is phylogenetically inclusive (holophyletic)—with the caveat that all included sub-groups are not going to be equally related. If such can be determined, then, armed with this “phylogenetic knowledge,” we would perhaps next seek to assess which name is descriptively most appropriate, given the “special biology” of the group that it is desired to recognize. After answering biological questions, one would presumably want to determine how properly to compose this name, including the proper name-ending. Again, in these matters, there is no effective counsel from the ICBN at kingdom level. This seems ironic, if not a full-blown “Catch-22,” in that one is bound into the botanical code for guidance for naming, but there is virtually no guidance (in the case of kingdoms). Cavalier-Smith and Chao (1996) alluded to inconsistencies (confusion, or lack of instruction as the case may be) in the botanical code concerning the establishment of names at higher ranks. As suggested (e.g., Blackwell, 2008, and above), such situations could be addressed more forthrightly if there were a unified code of nomenclature, with clear rules for naming higher categories—including kingdoms.

DISTINCTION OF PROPOSED KINGDOMS: CHROMISTA VS. STRAMINIPILA

Whereas Chromista and Stramenopiles, both, are now rather well-known and often accepted names/groupings, usage of the name Chromista is slightly longer standing—and Chromista is the more inclusive grouping. The kingdom Chromista was formally proposed by

Cavalier-Smith (1981), who subsequently (1986, 1989) provided more thorough expositions. The original Latin diagnosis by Cavalier-Smith (1981) emphasized: tubular mitochondrial cristae, chloroplast endoplasmic reticulum (complex plastids, with extra envelope membranes), and the presence of tubular mastigonemes (tubular "hairs") on at least one flagellum (cilium). The expansive content of kingdom Chromista (Cavalier-Smith, 1986, 1989) encompassed three presumably related phyla: **I.** Cryptophyta or "Cryptista" (the cryptomonads or cryptophyceans); **II.** Heterokonta: including, **A.** the "Ochrista" or chromophytous algae, such as chrysophytes, synurophytes, pedinellids, dictyochophytes (silicoflagellates), xanthophytes (tribophytes), eustigmatophytes, raphidophytes, phaeophytes, and bacillariophytes; **B.** "Pseudofungi," i.e., the Oomycetes, Hyphochytriomycetes, and the somewhat more protozoan-like labyrinthulids and the related thraustochytrids; and **C.** certain "protozoa," such as the bicoecids (or bicosoecids) which Cavalier-Smith (1986) first recognized as a group, lacking plastids, under the Ochrista; and, finally, **III.** Haptophyta or "Haptomonada" (the prymnesiophytes, which include the stratigraphically significant coccolithophorids). As discussed below, the grouping which came to be known as "Stramenopiles" (Patterson, 1989) is generally equivalent to phylum II (Heterokonta) of Cavalier-Smith's "Chromista."

The (usually) two flagella of heterokont chromistan motile cells are heterodynamic, with quite different actions or "beats" (cf. Sleigh, 1989). Tubular mastigonemes (typically tripartite, flagellar hairs)—sometimes known as "retronemes" (a more specific, functional term), because these generate a reversal of flagellar thrust (Cavalier-Smith, 1986, 1989; Round, 1989)—are found on the more anterior of the two subapical or lateral flagella (or on the only flagellum in some cases). These distinct, composite (three-tubulate) mastigonemes were determined to be often associated with a distinct organization of the flagellum-to-basal-body ultrastructure, viz. the "transitional helix" (cf. Patterson, 1989; Preisig, 1989). Such heterokont Chromista are, as indicated, known as "Stramenopiles"—in reference to the tubular mastigonemes (cf. Patterson, 1989). Photosynthetic representatives of Chromista typically have chlorophylls "a" and "c," but not "b" (Cavalier-Smith, 1986; Jeffrey, 1989). More than one form of chlorophyll "c" may be present (Jeffrey, 1989). Distinctive carotenoids

frequently occur (Bjørnland and Liaaen-Jensen, 1989), imparting often (not exclusively) a golden-brown pigmentation to the plastids.

Additional groups of stramenopilous (heterokont) algae, that is, groups added to the list of Ochrista (or chromophytous Chromista) since Cavalier-Smith's treatments (1986, 1989), include phaeothamniophytes, bolidophytes, and pelagophytes (see e.g., Blackwell and Powell, 2000, p. 71). Horn et al. (2007) proposed Synchronophyceae as a new class for an amoeboid "heterokontophyte" with a peculiar plastid complex. Relationships of certain other groups—such as the opalinids and the proteromonads—to Chromista have been postulated, but are equivocal (discussed in Blackwell and Powell, 2000). Regardless, there is no question that the Chromista (*sensu* Cavalier-Smith, 1986) are a diverse assemblage, including forms ranging from diminutive golden algae, diatoms, and large brown algae, to water molds, slime-nets (labyrinthulids), and related "protozoa" (see website: <http://www.ucmp.berkeley.edu/chromista/chromista.html>). A preliminary cladistic analysis of Chromista was presented by Williams (1991), supporting relationships among heterotrophic (including pseudofungal) and autotrophic (chromophytous algal) members.

The Kingdom (Regnum) name "Chromista" (Cavalier-Smith, 1981) apparently stems *in part* from the Division (apparent Class) name, "Chromophycées" (Chadefaud, 1950)—cf. Christensen (1989). Other related names, though, are more directly equivalent to Chadefaud's name, such as "Chromophyta" (Christensen 1962, 1989). Christensen (1989) formally proposed Division Chromophyta, including a Latin diagnosis (emphasizing the *absence* of chlorophyll *b*). Cavalier-Smith (1986) had earlier, however, validated Chromophyta as a Subkingdom name (Latin diagnosis high-lighting the tubular mastigonemes and tubular mitochondrial cristae). Subkingdom Chromophyta (name meaning "colored plant") represents a difficult concept, in that—being "above" phylum Heterokonta in Cavalier-Smith's classification—it includes both chromophytous algae (which typically have colorful plastids) and pseudofungi (which lack plastids, and therefore often lack pigment or special color as well). According to Cavalier-Smith (1986), Subkingdom Chromophyta is typified by genus *Chromophyton*. There is indeed a "chrysophyte" genus name *Chromophyton* Woronin (Bot. Zeit. 38: 625, 1880), cf. *Index Nominum*

Genericorum. However, it is not clear in the ICBN (2006) that automatic typification applies to Subkingdom and Kingdom level (see, for example, Article 16.2); nor is it clear, even if it did, that “Chromophyta” would be the correct name-form based on *Chromophyton* (see Christensen, 1989, but compare his view with Articles 10.7 and 16.4). In any case, the nomenclatural propriety of Chromophyta (be it considered a divisional or a subkingdom name) does not directly affect the legitimacy of Cavalier-Smith’s kingdom name Chromista—especially if Chromista is viewed as primarily a descriptive name (Article 16.1), viz. “colored protists.”

Seemingly more pertinent to the question of whether Chromista should be the kingdom name recognized is that, prior to Cavalier-Smith’s (1981) Chromista, Jeffrey (1971) had proposed a similar (if somewhat more polyphyletic) Kingdom, the “Chromobiota.” Jeffrey (1982), however, later modified this to a more monophyletic, Subkingdom grouping, the “Chromobionta”—inserting an “n” into the name—a grouping more or less equivalent to phylum Heterokonta of kingdom Chromista (cf. Cavalier-Smith, 1986). But, Jeffrey provided no Latin diagnosis for either name, Chromobiota or Chromobionta, leaving them (technically) nomenclaturally invalid (cf. Article 36.1, 36.2). Regardless, since priority is only a recommendation above the rank of Family (Recommendation 16B, even this not clearly applying to Kingdom), and since names (in specific reference here to subkingdom names) have no necessary priority outside of their original ranks (Article 11.2), there is no obligation (for one reason, or another) to employ Jeffrey’s (or Christensen’s, see above) name(s) at Kingdom level. Hence, the Kingdom name Chromista may be recognized, and attributed to Cavalier Smith (1981), with no requirement to reference other, perhaps similarly intended, names. Again, whether one can argue (spuriously, I believe) that Kingdom “Chromista” is an automatically typified name, based on the stated typification of Subkingdom Chromophyta by Cavalier-Smith (1986)—see paragraph above—is a matter of debate. However, this point is relatively moot to name selection, given the lax position of the ICBN on priority at higher levels (especially kingdom). Specific rules for naming Kingdoms (and Subkingdoms) would be a helpful addition to the botanical code—or better still, to a future, unified code of biological nomenclature.

In consideration of the fact that Chromista, as outlined by Cavalier-Smith (1986), constitutes a diverse assemblage, somewhat vaguely defined—the name seeming to emphasize the “algal” or plastid-bearing representatives more than the “fungal” members—Patterson (1989) suggested that “core chromophytes” (primarily the heterokont assemblage of chromophytous algae), along with related pseudofungal and “protozoan” representatives, be recognized (informally, at the time) by a more uniformly appropriate name, “Stramenopiles.” Patterson (1989) coined this name (meaning, literally, “straw hairs”) emphasizing the distinctive, lineage-defining, tubular flagellar hairs (i.e., the composite, tubulate mastigonemes) possessed by members of this group. A more precise group is thus suggested by the name Stramenopiles than is the case with the more inclusive Chromista, although the overlap of these two large groupings is very substantial. As has been indicated, Stramenopiles correspond to the phylum Heterokonta (Cavalier-Smith, 1986) of kingdom Chromista. Haptophytes and Cryptomonads (both groups included in the Chromista, cf. Cavalier-Smith, 1986) are excluded from Stramenopiles (*sensu* Patterson, 1989, and later publications, e.g., Blackwell and Powell, 2000). “Algal” representatives of Stramenopiles—the ochristal heterokont groups (goldens, browns, xanthophytes, diatoms, pelagophytes, eustigmatophytes, etc.) listed previously—have informally been referred to as “stramenochromes” (Leipe et al., 1994)—acknowledging the tubular mastigonemes as well as the often colorful plastids. There appears to be no comparable (“strameno---”) designation for pseudofungal or “protozoan” members of this heterokont grouping.

Ultrastructural studies on Chromista (particularly Stramenopiles)—such as of the flagellar apparatus and transition zone, as well as the flagellar hairs—proved useful in establishing relationships of member groups, among (and between) Ochrista (chromophytous algae) and Pseudofungi (e.g., Hibberd, 1979; Moestrup, 1982; Beakes, 1989; Cavalier-Smith, 1989; Patterson, 1989; Preisig, 1989; O’Kelly, 1989; Owen et al., 1990a,b; Andersen, 1987, 1991). Molecular confirmation of the “unity” of Stramenopiles (or organisms that would come to called such) was established, among others, by Gunderson et al. (1987), Ariztia et al. (1991), Bhattacharya et al. (1992), Leipe et al. (1994), Wee et al. (1996), and Honda et al.

(1999). Based on morphological and molecular information, the Stramenopiles came to be viewed as a kingdom or kingdom-like category (i.e., a “crown” group) by Leipe et al. (1994), Blackwell and Powell (1995, 1999), Alexopoulos et al. (1996), Van de Peer and De Wachter (1997), and Sogin and Silberman (1998). The name “Stramenopile” (originating, as indicated, with Patterson, 1989) found its way into textbooks of phycology (e.g., Lee, 1999), and Stramenopiles were recognized in selected biological diversity texts, e.g., Barnes (1998). In their introductory college biology textbook, Campbell et al. (1999) put forward this group as a “candidate kingdom,” employing a formalization of the name, “Stramenopila.” Alexopoulos et al. (1996) had earlier made use of “kingdom Stramenopila” in correctly asserting that organisms morphologically, nutritionally and ecologically thought of as “fungi” actually encompass more than one kingdom—Fungi, Stramenopila, and various Protist groups (or Fungi, Chromista, and Protozoa, cf. Beakes, 1998). Blackwell and Powell (2000) presented a detailed consideration of (and support for) the phylogenetic integrity of the overall stramenopilous assemblage. Ideas on the filiation of the numerous member groups of Stramenopiles are found in Sogin and Patterson (1995, Tree of Life Web Project) and Blackwell and Powell (2000). Some authors have continued to use the name Stramenopiles (Reyes-Prieto et al., 2007), while others (e.g., Baldauf et al., 2000) recognized the stramenopile grouping, but employed other names—in this latter case the generally equivalent category, Heterokonta, of Cavalier-Smith (1986).

In spite of the recognition mentioned above, it was apparently not until the book, *Straminipilous Fungi*, published by Dick (2001), that Stramenopiles were formally proposed (Latin diagnosis presented) as a Kingdom—viz., kingdom “Straminipila.” Dick’s circumscription appears primarily to include pseudofungal organisms (By whatever names employed, it is these that are enumerated)—although he spoke (pursuant to the diagnosis) of “coevolutionarily linked endosymbiont characters,” including plastid and chlorophyll features, in seeming reference to “algal” representatives. Dick does note in introductory discussion that “biflagellate fungi” and chromophyte algae, as well as labyrinthulids for example, are unified by the “straminipilous flagellum”—i.e., the anterior “tinsel” flagellum of previous discussion, bearing composite, tubular mastigonemes. It is plausible that Dick

intended to include chromophytous algae (and bicocoids) by his statement in the diagnosis concerning “organisms that originally possessed, or evolved to possess” such features as heterokont flagella and straminipilous scales. In any case, it seems a little strange that Dick refers to straminipilous “fungi,” since, as he himself notes, these are not true fungi. As for nomenclatural detail, indication by Dick of the holotype of kingdom Straminipila, as phylum Heterokonta Cavalier-Smith [1986], is unnecessary since “Straminipila” is a descriptive, not a typified, name (cf. Article 16.1, ICBN)—and the type method does not otherwise apply above the rank of Family (compare Articles 7.1 and 16); requirement for citation of type for validation purposes is, in fact, primarily at genus level or below (Article 37). Dick’s spelling of the name of this kingdom is unique, viz. “Straminipila.” He not only altered the spelling to “Straminipila” (from, presumably, “Stramenopila”), he listed his name (alone) as author of the kingdom (regnum). This assignment of authorship by Dick (to himself) is technically correct, although, as has been indicated, Patterson (1989) originated the informal name (and the concept of) “Stramenopiles,” and others (as mentioned), prior to Dick, used the name Stramenopila. Dick’s alteration of the connecting vowel in the name (from “o” to “i”) is appropriate (cf. Stearn, 1983, p. 269). However, there was nothing incorrect about the spelling of the second syllable of the name “Stramenopila” (based on Latin, *stramen*), as given in the kingdoms listed by Alexopoulos et al. (1996) and Campbell et al. (1999)—although, these were, of course, not intended as formal kingdom proposals (no Latin diagnoses provided). If *stramen*, a noun, is (in the name Straminipila) employed adjectivally (cf. Stearn, 1983, p. 267), i.e., deriving from *stramineus* (cf. Simpson, 1968), then Dick’s spelling (Stramin-i-pila) would be acceptable. Dick (2001), however, indicated the etymological derivation to be from *stramen* [the noun]—this being equivalent to Patterson’s (1989) original usage. But, even if one accepts Dick’s kingdom, name and spelling, Straminipila, it would not seem inappropriate to cite authorship as Patterson ex Dick (cf. Article 46), since Patterson (technicalities aside) generated the name basis and originated the construct of what would become this “kingdom.” And, if kingdom Straminipila is recognized (regardless of spelling), it should be rendered convincingly more inclusive (i.e., formally emended, cf. Recommendation 47A)—in the sense of Stramenopiles as circumscribed, for example, by Patterson (1989), Leipe et al. (1994)

and Blackwell and Powell (2000)—so that “chromophytous algal” and “protozoan” member groups are definitively included (listed, and accorded equal importance to the “pseudofungal” representatives emphasized by Dick, 2001). This suggested inclusiveness is especially pertinent given recent evidence of (not plastids but) plastid-associated genes in Oomycetes (cf. Tyler et al., 2006; Bailey, 2008; Sanchez-Puerta and Delwiche, 2008), indicating further relationship of algal and pseudofungal representatives of stramenopiles. However, formal emendation becomes truly important only if Straminipila is selected as the kingdom to best represent heterokont chromistans—rather than simply recognizing this group as, for example, phylum Heterokonta of kingdom Chromista (Cavalier-Smith, 1986). If accepted, “Stramenipili”—the first half of the name based on the Latin noun, *stramen* (straw), and the second half based on the Latin noun, *pilus*, *pili* (hair, hairs), cf. Simpson (1968)—might be a preferable spelling (to Dick’s “Straminipila”), and more comparable to Patterson’s original, informal “Stramenopiles.” Such orthographic changes are permitted (if justifiable) by the ICBN without invalidation of the standing name, authorship or date of publication (cf. Articles 32.7 and 60.1), i.e., the validating author would still be Dick (2001). And, recall (first paragraph of text following Key Words), there is no rule (cf. ICBN) that kingdom names must end in “-a.” The ending, “pili” (of Stramenipili) is not only permissible, it would unambiguously satisfy the requirement that the name be treated as a noun in the plural (Article 16.1). However, such points concerning spelling (as those concerning emendation) fade in significance if Straminipila (Dick) is not favored as a kingdom over Chromista.

It might be assumed that the name Straminipila (or Stramenopila, or Stramenipili—depending on interpretations of etymology and orthography) should be selected for the kingdom in question, because of the relatively cohesive phylogenetic circumscription of this group (cf. Patterson, 1989; Leipe et al., 1994; Blackwell and Powell, 1999, 2001; Blackwell, 2004). Stramenopiles are restricted to organisms that are actually “heterokont,” implying the presence of composite (usually three-parted), tubular mastigonemes on the more forward of two flagella (or the only flagellum in some cases). Such unique flagellar appendages are considered lineage-defining (cf. Leipe et al., 1994; Blackwell and Powell, 2000). Recent evidence has

indicated that tubular mastigonemes of stramenopiles (Yamagishi et al., 2007, studying *Ochromonas*) are not only structurally but compositionally different from those of the simple mastigonemes of green algae, such as *Chlamydomonas*. In the interest of avoiding semantic confusion, proteins composing tubular mastigonemes (the mastigonemes, of course, externally attached to flagella) appear to be unrelated to tubulin proteins of actual microtubules (of which flagella, and certain other cytoskeletal elements, are composed). But, regardless of the seeming distinctiveness of Stramenopiles, there are complications. In the more broadly cast kingdom, Chromista, additional groups are included and must be considered—viz., the cryptomonads and the haptophytes—even if these have been placed in different phyla, or in some cases subkingdoms, from heterokonts (Cavalier-Smith, 1986, 1989). Pursuant to Cavalier-Smith's initial expositions, certain authors have apparently found haptophytes and cryptomonads to be relatively unrelated to the heterokont assemblage (i.e., to Stramenopiles)—see, for example, Daugbjerg and Andersen (1997) concerning haptophytes, and Van de Peer and De Wachter (1997) regarding cryptomonads. This viewpoint (including the consideration that "Chromista" was possibly too broad of a construct) would seem to support recognition of a separate kingdom Straminipila (as by Dick, 2001) for truly heterokont organisms. Other authors (e.g., Bhattacharya and Medlin, 1995; Cavalier-Smith, 2002), however, have appeared to indicate a degree of relationship between heterokonts (stramenopiles), cryptophytes and haptophytes—and if this is so, a kingdom Straminipila would perhaps be too limiting, and a broader construct (Chromista) would be favored. So, how does one decide whether major chromistal groups are substantially related?

The monophyly of the pseudofungal groups of chromistans has not (in recent times) been substantially in question (cf. Blackwell and Powell, 2000). Now, in consideration of "algal" representatives, information has come to light to suggest that there was a common, eukaryote/eukaryote (i.e., "secondary") endosymbiosis—involving a red algal endosymbiont—connecting (through common plastid ancestry) the cryptomonad, haptophyte and heterokont "algae"—cf. Cavalier-Smith (1992, 2002), Delaney et al. (1995), Delwiche (1999), Palmer (2003), Bhattacharya et al. (2004), Keeling (2004), Li et al. (2006), and Reyes-Prieto et al. (2007). Possibly, more than one such

major secondary (or even a tertiary) endosymbiotic event was involved (Sanchez-Puerta and Delwiche, 2008). But in any event, the general consensus of references cited above (among others) suggests that the diverse “algal” (i.e., plastid-containing) representatives of the Chromista (*sensu* Cavalier-Smith, 1986) are also, broadly, monophyletic (that is, with regard to origin of their plastids, i.e., involving the same, an identical, or a very similar, secondary endosymbiosis). The kingdom Chromista, as conceived by Cavalier-Smith (1981, 1986, 1989), thus represents not only a larger grouping of organisms (than Straminipila), but possibly one that can still be viewed (by some measures at least) as monophyletic as well (Cavalier-Smith, 2002)—even if not as obviously (clearly definably) monophyletic as the Stramenopiles. In other words, based on recent knowledge of plastid evolution (e.g., Keeling, 2004; Reyes-Prieto et al., 2007), it is not unreasonable to consider the Chromista as the more holophyletic—if plainly the circumscriptively looser and phylogenetically more diverse—of the two assemblages (Chromists and Stramenopiles). Therefore, if putative holophytesis (at least in the sense of containing a greater number of paraphyletic groups, cf. Bhattacharya et al., 1992; Schuh, 2000) is the guideline for kingdom selection, the nod would seem to go, for now, to Chromista (over Straminipila); however, the matter cannot be considered finally settled.

IF NOT STRAMINIPILA, IS CHROMISTA THE BEST REMAINING OPTION?

If deciding not to use the name Straminipila (or a related spelling) for this assemblage—because it is not the most encompassing group—then is one left with Cavalier-Smith’s (1981) name Chromista, with its attendant broad circumscription? Perhaps so, but there are additional problems. The name “Chromista” is without universal applicability of meaning, even within the heterokont assemblage. “Chrome” (Greek/Latin: *Chroma*, *Chromus*) implies the presence of color or pigment; principally, it came to connote the “brown” line of algae (Round, 1989), as distinct from “green” or “red” algae. However, as noted by Cavalier-Smith (1986) and Round (1989), not all members of Chromista are pigmented. Pseudofungi, such as Oomycetes and hyphochytrids, and pseudofungal/protozoan representatives such as labryrinthulids (as well as the “more protozoan” bicoecids), are without

actual plastids (even though plastid genes may be present in Oomycetes, cf. Bailey, 2008). Also, the name “chrome” is vague in meaning (simply, “color”)—not precise given the various hues encountered in representative chromophytous algal groups (brown, golden, golden-brown, reddish-brown, yellow-green, almost grass green, and even other hues). And, any suggestion of “Protista” in the name Chromista (viz. “chrome-ist” abridging “chrome-protist”) is superannuated, since the hodgepodge “protist” or “protoctist” kingdom (Haeckel, 1866; Whittaker, 1969; Margulis, 1981; Corliss, 1984) is no longer phylogenetically tenable (Cavalier-Smith, 1987, 1993; Corliss, 1994; Blackwell and Powell 1995, 2001). However, a name such as Chromista is not to be rejected because it is not compellingly descriptively appropriate (Article 51, ICBN, 2006). For that matter, the meaning of “Chromista” is not entirely inappropriate, being applicable generally to the chromophytous algal representatives (although there are colorless chrysophytes, e.g., as investigated by Belcher and Swale, 1972). Furthermore, usage of the name Chromista (in the sense of Cavalier-Smith, 1986, 1989) has been steadfastly inclusive of rather diverse groupings that continue to seem suitable for inclusion (on plastid evidence, for example, cf. Keeling, 2004). Before finally accepting this kingdom name, however, it should be asked if other legitimate, descriptively appropriate names are available for use?

A kingdom name that preceded Cavalier-Smith’s (1981) Chromista was Ochrobionta (Edwards, 1976). Edward’s “Ochrobionta” is loosely equivalent to the “Ochrista” (recognized later by Cavalier-Smith, 1986), and to “Ochrophyta” (Cavalier-Smith, 1997)—viz., “Ochrophytes” (Graham and Wilcox, 2000). In other words, Ochrobionta (Edwards) is composed mainly of what would come to be viewed as the chromophytous algal component of kingdom Chromista (including though, in Edward’s view, cryptophytes in addition to ochristal chromophytes). The kingdom name Ochrobionta, however, would not now be considered acceptable for several reasons. For one thing, “Ochrobionta” was not validly published (no Latin diagnosis). Secondly, organisms belonging to the pseudofungal group of heterokont chromistans were not covered by Edward’s construct (Ochrobionta). As a third point, dinoflagellates (“Pyrrhophyta”) were included in Ochrobionta by Edwards—not a desirable placement (as presently understood), since dinoflagellates, regardless of ultimate

potential (multiple) plastid connections (cf. Keeling, 2004), are probably not as immediately related to chromophytous algae of the Chromista as they are to (other) Alveolate Protozoa (cf. Cavalier-Smith et al., 1995; Hausmann and Hülsmann, 1996; Blackwell and Powell, 2001; Yoon et al., 2005). Finally, Edwards (1976) spoke of “a preponderance of carotenoids over chlorophylls” in members of his Ochrobionta, seeming to downplay the role of chlorophyll which is still the primary photosynthetic pigment in these organisms—and, some chromophytes are indeed decidedly greenish in coloration, especially certain members of the Xanthophyceae (a fact which Edwards, 1976, acknowledged). A proposed kingdom mentioned previously, Chromobiota Jeffrey (1971), though similar to Edward’s Ochrobionta, did include some pseudofungi (i.e., as presently known). However, Jeffrey’s Chromobiota is otherwise beset with the same circumscriptive and nomenclatural problems as Edward’s “kingdom”—e.g., inclusive of dinoflagellates, lacking Latin diagnosis. Edward’s (1976) apparently partially patterned his kingdom (Ochrobionta) after Jeffrey’s (1971) Chromobiota.

As for other “kingdom” name possibilities, a perhaps more serious candidate, Heterokonta, would appear to be available, and some recent authors (e.g., Baldauf et al., 2000) have employed this name. The name “Heterokonta” is descriptively applicable to the distinct, heterodynamic flagella—one forwardly directed pleuronematic (“tinsel” or “hairy”) flagellum (bearing tubular, reverse-thrusting mastigonemes), and one, sometimes trailing, smooth, whiplash flagellum (with a more typical flagellar motion)—of heterokont chromistan groups that are biflagellate (the majority), cf. Moestrup (1982), Cavalier-Smith (1986), Van den Hoek et al. (1995). However, there are problems. “Heterokonta” was formally established—Latin diagnosis focusing on tubular mastigonemes of the anterior flagellum of heterokonts: “algal,” “pseudofungal,” etc.—as a Phylum (Division) name by Cavalier-Smith (1986), not a Kingdom name. And, as pointed out, a name does not have priority outside its own rank (Article 11.2, ICBN)—even if we allow that priority carries any force at these upper ranks (kingdom, subkingdom, phylum, subphylum, etc.)—although a given descriptive name may in fact be used at different ranks (Article 16.1). Earlier, Cavalier-Smith (1978) had informally (no Latin diagnosis) suggested “Heterokonta” as a kingdom name—but for a

heterogeneous assemblage, including not only chromophytous algae and Oomycetes, but also chytrids (which are true fungi), Myxomycetes (i.e., slime molds, which are Protozoa), and Foraminifera (also Protozoa); yet, this unwieldy grouping did not include cryptophytes (which are usually considered to be chromistans). Subsequent to better understanding, Cavalier-Smith (1986) abandoned Heterokonta as an overly diverse kingdom concept, in favor of the more circumspect divisional usage of the name (i.e., for a grouping generally equivalent to what would subsequently be termed Stramenopiles, cf. Patterson, 1989; Leipe et al., 1994; Blackwell and Powell, 2000). Later, perplexingly, Cavalier-Smith (cf. 1995, 1997) “raised” Heterokonta to “infrakingdom” (= subkingdom?, cf. Article 4.2). In further complication, Cavalier-Smith’s (1986) phylum Heterokonta, though well-defined phylogenetically, is readily confused with the pre-existing (much older) name Heterokontae (cf. Luther, 1899; Pascher, 1925; Fritsch, 1935). Heterokontae, in the sense of these latter authors, is generally equivalent to the algal Class, Xanthophyceae (Tribophyceae, cf. Ott, 1982); the name (“Heterokontae”) thus applies primarily to only a limited subset of Heterokonta (*sensu* Cavalier-Smith, 1986).

Another (somewhat older) version of the phylum name “Heterokonta” (Cavalier-Smith, 1986) is “Heterokontophyta” (Van den Hoek, 1978)—likewise used as a phylum name (or seemingly so) by several authors (e.g., Moestrup, 1982, 1992; Van den Hoek et al., 1995; Horn et al., 2007; Sanchez-Puerta and Delwiche, 2008). “Heterokontophyta” has been applied more to algal than pseudofungal representatives of heterokonts. Cavalier-Smith and Chao (1996), however, pointed out that the name Heterokontophyta was questionably validly published, and favored use of Ochrista instead—It should be noted, though, that Ochrista (Cavalier-Smith, 1986) was published as a subphylum (subdivisional) name, not a phylum (division) name. Regardless, Lee (1999) used Heterokontophyta, *de facto*, in general correspondence to subphylum Ochrista of Cavalier Smith (1986). No matter the exact previous rank, name permutation, or usage employed, a pragmatic problem with a potential *kingdom* Heterokonta (or Heterokontophyta) is that not all chromistans are morphologically “heterokont,” as the term is precisely defined—implying not just flagella of (often) unequal length, but two structurally and functionally different flagella on the same cell (cf. Van den Hoek et al., 1995,

glossary, re: “heterokont zoids”). “Heterokonta” was formally founded on this flagellar distinction (including the presence of tubular mastigonemes on the forward flagellum, cf. Cavalier-Smith, 1986). “Heterokonta” is, as a consequence, not an inclusive enough category for the entire chromistan assemblage. For example, Haptophytes usually have two, similar, “apical,” whiplash flagella, plus a “haptonema” (a central, superficially “flagellum-like,” sometimes coiled, appendage—cf. Sleigh, 1989; Van den Hoek et al., 1995). Haptophytes are *not* Heterokonts, yet they still appear to qualify as Chromistans (based on knowledge of plastid evolution, cf. Bhattacharya et al., 2004; Keeling, 2004). “Heterokonta” (*sensu* Cavalier-Smith, 1986) thus constitutes, even in broadest usage, too narrow of a kingdom concept to encompass all chromistans, as historically and presently recognized.

In final analysis, “Chromista” (as conceptualized by Cavalier-Smith, 1981, 1986, 1989) remains the most applicable name for the over-all group of heterokont *and potentially related* organisms discussed herein as a kingdom (Reference the listings in the first and third paragraphs of the preceding section: “Distinction of Proposed Kingdoms...”). The main consideration that might alter future acceptance of kingdom Chromista is not the appropriateness of the name, or the potential “challenge” of other competing names, but rather the question of the degree of relationship of the somewhat disparate, major member (chromistal) groups, that continue to be included (discussed below).

CONCLUDING POINTS, CURRENT VIEWS, AND CONCERNS (Not only Kingdoms and their delimitation, but “Supergroups”)

Though not representing an overwhelming consensus, the balance of currently available information indicates that the kingdom name Chromista Cavalier-Smith (1981; see also 1986, 1989) is the best option for proper application to, and implicit circumscription of, the presumed reasonably holophyletic assemblage of chromophytous algal, pseudofungal, and related primitive protozoal organisms discussed herein (see again, “Distinction of Proposed Kingdoms...” section, listings in first and third paragraphs). The Stramenopiles (as delineated, for example, in Patterson, 1989; Leipe et al., 1994; and Blackwell and

Powell, 2000) are probably best viewed, presently, as constituting a major phylum of kingdom Chromista. It is possible (if one so wished) to use the kingdom name Straminipila Dick (2001)—by whatever spelling (discussed previously)—as a phylum (division) name, rather than Heterokonta Cavalier-Smith (1986), since priority generally does not apply above family rank (Article 11.1, ICBN, 2006), and since descriptive names (such as Straminipila) may be used, unchanged, at different ranks (Article 16.1). The suggested endings, “-phyta” or “-mycota,” for divisional names in the botanical code are, in the case of Straminipila (or Heterokonta, for that matter), not only inappropriate, but constitute merely a recommendation (16A.1)—firm rules for properly establishing a name such as this are lacking. Again, in these instances, one could wish for a unified code with well-reasoned, unambiguous rules for names of “higher” ranks. In any case, though, the possibility still exists that Straminipila Dick (2001), if emended to be more clearly defined and formally inclusive—e.g., as concerns chromophytous heterokont (i.e., certain “chromophytous algal”) groups—could eventually be accepted as a kingdom-level category, perhaps even replacing the more heterogeneous Chromista. However, this replacement would come to bear only if seemingly authenticated relationships of non-stramenopilous chromistan groups to stramenopiles are *not* sustained (see discussions in Harper et al., 2005 and Sanchez-Puerta and Delwiche, 2008). But, should future evidence indicate that cryptomonads and haptophytes (prymnesiomonads) are no longer tenable as members in an assemblage containing stramenopiles (i.e., within the Chromista), these groups would possibly revert to temporary systematic placement in the “catch-all” kingdom Protozoa (cf. Blackwell and Powell, 2001).

Some recent authors have indeed adopted (or re-adopted, as the case may be) usage of “Chromista” in the sense of a kingdom name (e.g., Bhattacharya et al., 2004; see also the website: <http://www.ucmp.berkeley.edu/chromista/chromista.html>). Given the “super-groups” of organisms now recognized (Cavalier-Smith, 1999; Palmer, 2003; Bhattacharya et al., 2004; Keeling, 2004; Parfrey et al., 2006), Chromista (as compared with the more restrictive grouping Stramenopiles) is the kingdom which appears more broadly suited (further discussed below) for membership within the supra-kingdom grouping, Chromalveolata (cf. Cavalier-Smith, 1999; Adl et al., 2005;

Reyes-Prieto et al., 2007)—an assemblage encompassing not only chromists, but also the related alveolate protozoa, i.e., dinoflagellates, ciliates and apicomplexans (cf. Bhattacharya and Medlin, 1995; Hausmann and Hülsmann, 1996; Blackwell and Powell, 2001; Cavalier-Smith and Chao, 1996; Cavalier-Smith, 2002).

Relationships between certain Chromists and Alveolates (still somewhat equivocal, cf. Sanchez-Puerta and Delwiche, 2008) were established, among others, by Cavalier-Smith et al. (1995), Van de Peer and De Wachter (1997), and Sogin and Silberman (1998)—a connection (based partly on plastid genetics, cf. Cavalier-Smith, 2002; Keeling, 2004) that has, so far, generally held up under scrutiny (Adl et al., 2005; Parfrey et al., 2006). However, such a phylogenetic relationship is perhaps one that is chimaeric (cf. Corliss, 1994; Cavalier-Smith, 2002; Parfrey et al., 2006), not necessarily taking the composite organism (“holobiont,” cf. Mindell, 1992) into account. Recent evidence (Harper et al., 2005; Sanchez-Puerta and Delwiche, 2008), including evidence from genes *additional* to those involved with plastids, supports a closer relationship of alveolate protozoa with heterokont members of the Chromista (i.e., with stramenopiles) than with either of the other putative chromist groups: haptophytes (prymnesiomonads) or cryptomonads (cryptophytes)—see also Adl et al. (2005). Among chromistan organisms, thus, there may be only a distant overall (host-cell?) relationship between stramenopiles (true heterokonts) and either cryptomonads or haptophytes. On the other hand, there is some evidence of relationship between these chromistan groups: for example, the tubular flagellar hairs of cryptophyceans are similar (although bipartite, rather than tripartite) in morphology to those of true heterokonts (Moestrup, 1982; Cavalier-Smith, 1989); also, there is possibly a sibling relationship between cryptomonads and haptophytes (Sanchez-Puerta and Delwiche, 2008); and, some relationship of large-subunit (28S) cytoplasmic ribosomal RNA was indicated between chromophytous algae [stramenopiles] and haptophytes (Perasso et al., 1989). In any case, considering evidence pro and con, the recognition of Heterokonta, Cryptophyta, and Haptophyta as quite distinct phyla—yet these encompassed *within* the Chromista (Cavalier-Smith, 1986, 1989)—can be viewed as an assessment (by Cavalier-Smith) that was probably on target. As pointed out by Harper et al. (2005) and Sanchez-Puerta and Delwiche (2008),

more data is needed before final establishment of membership of the Chromalveolata (and final re-establishment of the Chromista, in my view). Both support and doubt have been expressed concerning the “chromalveolate hypothesis” (see Palmer, 2003; Adl et al., 2005; Harper et al., 2005; Li et al., 2006; Sanchez-Puerta and Delwiche, 2008)—i.e., concerning whether this very large grouping can truly be viewed as (even generally) monophyletic. Nonetheless, inclusion of Chromists and Alveolates in a common super-group is for the time-being reasonable (Adl et al., 2005; Yoon et al., 2005; Li et al., 2006), and comparable to inclusion of Fungi and Animalia in the Opisthokonts (Unikonts). Both “supergroups,” Chromalveolata and Opisthokonta, are arguably tenable based on selected morphological and molecular grounds (cf. Keeling, 2004; Adl et al., 2005; Parfrey et al., 2006; Reyes-Prieto et al., 2007).

Given the above, the final question on the propriety of kingdom name selection (e.g., Chromista) concerns how well the kingdom fits (including considerations of phylogeny) with other kingdoms (or kingdom components) in the context of larger, super-group assemblages now recognized (e.g., Keeling, 2004; Adl et al., 2005; Parfrey et al., 2006)—Amoebozoa, Opisthokonta, Rhizaria, Archaeplastida (Plantae), Chromalveolata, and Excavata. In the case of the super-grouping pertinent to the present paper—Chromalveolata (grouping Chromista with Alveolata)—the fit would appear to be relatively good, given improved, if still controversial, knowledge (especially plastid information, cf. Cavalier-Smith, 2002; Bhattacharya et al., 2004; Keeling, 2004; Reyes-Prieto, 2007) of phylogenetic relationships among major subgroups of these organisms. If it is eventually determined (see discussion in Adl et al., 2005; Harper et al., 2005; Sanchez-Puerta and Delwiche, 2008), however, that haptophytes and cryptomonads are sufficiently phylogenetically distant from alveolates (and stramenopiles) to be marginalized or even excluded from the “Chromalveolata,” then a new supra-kingdom name may need to be formulated (based on a more restricted grouping). This new name could possibly derive from a combination of the names Straminipila (representing a more precise circumscription than Chromista) and Alveolata, since truly heterokont organisms (stramenopiles) and certain alveolates appear substantially related (Van de Peer and De Wachter, 1997; Baldauf et al., 2000; Keeling, 2004; Harper et al., 2005; Reyes-

Prieto et al., 2007)—although the extent of their monophyly is not completely resolved (Sanchez-Puerta and Delwiche, 2008).

As evident from discussion, some recent authors (e.g., Keeling, 2004; Adl et al., 2005; Parfrey et al., 2006) have utilized quasi-formal “Supergroups” (six in total, see above) *instead* of Kingdoms. Adl et al. (2005) considered these largest groups “similar to traditional ‘kingdoms’”—however, they are not (as may be judged from Table 1 in Adl et al., 2005; and Figs. 1, 2 and 3 in Parfrey et al., 2006). Rather, such super-groups are *inclusive of* kingdoms, among other (not necessarily coequal) groupings; e.g., the super-group Opisthokonta includes Fungi, Metazoa (animals), Choanomonads, and Mesomycetozoa. Use of six supergroups in the sense of kingdoms (Adl et al., 2005), and a contemporaneous recognition of six (actual) kingdoms (Cavalier-Smith, 2004), is potentially confusing (different names are typically used for supergroups vs. kingdoms). Parfrey et al. (2006) made a limited attempt to sort the matter out. Possibly, future codes of nomenclature should serve not only to establish rules for naming kingdoms, but should take supra-kingdom assemblages into account as well. Kingdom and Supra-kingdom categories should be clearly distinguished. In point of fact, however, no supra-kingdom ranking (super-group category) is presently covered (or “allowed,” depending on one’s point of view) by any official code of nomenclature. A related question (scarcely raised to date) is, whether such “super-groups” should be called “Domains”?—as previously applied by Woese et al. (1990) to the most major prokaryotic groupings, Archaea and Bacteria—a question (re: the six largest eukaryotic clusters) for nomenclaturists of eukaryotes to decide. There are other options (in addition to “Domain”) for the appellation of super-groups (e.g., “Empire,” “Super-kingdom,” “Supra-kingdom”). Almost any option would seem preferable to the current designation of each of these largest assemblages by the informal, non-rank-identifiable term, “supergroup” (Parfrey et al., 2006). Regardless of which rank-category-name is ultimately selected, nomenclatural consistency would be desirable for such supervening taxonomic categories.

With continued emphasis on kingdom and supra-kingdom categories, it does not seem that we (as taxonomists) are necessarily proceeding just in the direction of “rankless” classifications (cf.,

Hibbett and Donaghue, 1998), toward a systematics of (only?) clades, not formal, named, taxonomic ranks. Whether stated or de facto, largely formal, hierarchical taxonomic systems still generally hold sway. Adl et al. (2005) indicated that the eukaryote cluster-group system they employed is “nameless” and (although they asserted the inherence of a “ranked systematics”) is a system “without formal rank designations”—i.e., a system “not [formally] constrained...” However, the vast majority of the taxonomic groups, including the six main clusters, in Adl et al. (2005) bear what appear to be formal names—three of six main cluster names stemming from works of Cavalier-Smith. Significantly, traditional formal names (for what might be known to some as phyla, classes, orders, families, etc.) continue to be used under the main cluster names (Adl et al., 2005)—de facto signifying particular ranks, even though ranks are not explicitly stated. The reference by Adl et al. is useful and comprehensive. However, the (rank-unidentified) name mix can be confusing, requiring that one search the context of particular names (helpfully, sources are provided). In some cases, perplexingly, names of different ranks are apparently considered to be at a comparable taxonomic level, e.g., *Schizocladia*, Synurales and Xanthophyceae (see Adl et al., 2005, p. 429).

The systematic descriptive enumeration of Adl et al. (2005) is unquestionably a valuable compilation; yet, it is not (I believe) the optimal, ultimate systematic approach to the higher level classification of eukaryotes. Though divided orderly into six clusters (of uncertain, though one would assume the *same*, “formal” rank), it is otherwise much of an “information-board presentation,” with items pasted from zoological, botanical, mycological, and protistological taxonomy. It would seem that, rather than attempting to piece together an *ad hoc* phylogenetic system such as Adl et al. (2005)—some potential nomenclatural pitfalls in *ad hoc* systems discussed in Blackwell (2002)—it would be prudent in the long run to take the trouble to render consistent the formal, upper-level nomenclature of eukaryotes, modifying not only the naming scheme employed but codes of nomenclature as well (an effort involving two at least partially different groups of workers—evolutionary systematists and nomenclaturists). In other words, the most desirable approach would be that of bringing both taxonomic/evolutionary schemata and nomenclatural codes into accord. If present codes of nomenclature are ill-tuned to the task, it

logically follows that efforts to rebirth a formal *BioCode* might be appropriate, given the perceived need by some (cf. Cavalier-Smith, 1978; Corliss, 1983, 1990; Patterson, 1986; Blackwell and Powell, 1999; Blackwell, 2008) for code reform. The *BioCode* draft (Greuter et al., 1996—minor revision done in 1997) did not meet with success, and was not adopted. It is pointless to debate its merits here; however, the draft *BioCode* did at least purport to add the rank “domain” (above kingdom) to existing ranks (of the botanical code). Perhaps future *BioCode* efforts (cf. Hawksworth, 2007), no matter what form the document might take, could give detailed consideration to kingdom, subkingdom, and supra-kingdom nomenclature—addressing what ranks should be recognized, and how names appropriate to these ranks should be formed. By so doing, perhaps a mechanism could be provided through which the most comprehensive clades, e.g., “crown clades” (cf. de Queiroz and Gauthier, 1992), could be formally recognized as “crown taxa” (cf. Van de Peer and De Wachter, 1997). If a future version of the *BioCode* does not prove to be the answer to supra-familial nomenclatural problems, development of a unified biological code of nomenclature (even if it be, initially, a minimal or “skeleton” code) should nonetheless be pursued, hopefully to the eventual outcome of acceptance by all factions involved. If, in the process, it is desired to accommodate particular elements of the unofficial *PhyloCode* (cf. Cantino, 2000; de Queiroz, 2006) in a new, formal, unified code of biological nomenclature—thereby enhancing the “phylogenetic capability” of nomenclature, while maintaining sound nomenclatural practice and eliminating undesirable competition between these two possible future codes—this would seem a reasonable and appropriate way to proceed.

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**GEOGRAPHIC VARIATION IN THE LEAF ESSENTIAL OILS
OF *HESPEROCYPARIS* (*CUPRESSUS*) *ABRAMSIANA*, *H.*
GOVENIANA AND *H. MACROCARPA*:
SYSTEMATIC IMPLICATIONS**

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ABSTRACT

The compositions of the volatile leaf essential oils of *Hesperocyparis abramsiana* and its putative subspecies (*Cupressus abramsiana* subsp. *locatellii*, *opleri*, *neolomondensis*, and *butanoensis*) are presented along with *H. goveniana*, *H. pygmaea*, and *H. macrocarpa*. Most of the putative subspecies of *H. abramsiana* oils contained large amounts of umbellulone (16-21.8%), while the putative *C. a.* subsp. *neolomondensis* (type 2 oil) and *H. pygmaea* contained the unusual terpene karahanaenone (18.4, 2.2%). With the possible exception of *C. abramsiana* subsp. *butanoensis*, none of the subspecies proposed by Silba (2003) was supported. *Phytologia* 91(2): 226-243 (August, 2009).

KEY WORDS: *Hesperocyparis abramsiana*; *Cupressus abramsiana* subsp. *butanoensis*, *locatellii*, *neolomondensis*, *opleri*; *Hesperocyparis goveniana*; *Cupressus goveniana* subsp. *gibsonensis*; *Hesperocyparis macrocarpa*; *Cupressus macrocarpa* subsp. *lobosensis*; *Callitropsis*, Cupressaceae; essential oil composition; taxonomy.

Silba (2003) recently described four new subspecies of *Cupressus abramsiana* Wolf: *C. a.* subsp. *locatellii* Silba, Eagle Rock, CA; *C. a.* subsp. *opleri* Silba, Bracken Brae, Santa Cruz, CA; *C. a.* subsp. *neolomondensis* Silba, Majors Creek, CA; and *C. a.* subsp.

butanoensis Silba, Butano Ridge, CA. In addition, Silba (2003) split *C. goveniana* Gordon and *C. macrocarpa* Hartw. into subspp. *gibsonensis* and *lobosensis*, respectively. Because these proposed new subspecies are morphologically rather indistinct, we collected samples of fresh foliage from five separate trees from all of the type localities from which we then extracted and analyzed the leaf volatile oils to gather additional genetic information.

Recent DNA sequencing of *Cupressus sensu lato* (Little et al., 2004, Little, 2006) demonstrated that the Western Hemisphere species form a well-supported clade quite separated from the Eastern Hemisphere cypresses. As a result, Little (2006) not only confined the genus *Cupressus* to the Eastern Hemisphere, he also used *Callitropsis nootkatensis* and its generic epithet for the Western Hemisphere cypresses and *Xanthocyparis vietnamensis*. Debreczy et al. (2009) later argued, on morphological grounds, that *Ca. nootkatensis* is a monotypic genus. Sequencing by Adams et al. (2009) of two additional nuclear genes and petN-psbM further supported the recognition of *Ca. nootkatensis* as a monotypic genus. Because *Callitropsis*, therefore, should not be applied to the Western Hemisphere cypresses, Bartel and Price in Adams et al. (2009) described a new genus, *Hesperocyparis*, for the Western Hemisphere cypresses (exclusive of *X. vietnamensis* and *Ca. nootkatensis*). However, when referring to Silba's subspecies, *Cupressus* is used throughout this paper to avoid creating any new name combinations.

The volatile leaf oil of *Cupressus macrocarpa* has been examined by several authors: Briggs and Sutherland (1942); Zavarin et al. (1971); Briggs and Kingsford (1974); Malizia et al. (2000); Floreani et al. (1982); Cool (2005); El-Ghorab et al. (2007); Manimaran et al. (2007). However, only Zavarin et al. (1971) and Cool (2005) examined oils from trees native to California. Zavarin et al. (1971) confined their analysis to the monoterpenes, and concluded that *Cupressus macrocarpa* was distinct in its leaf oil. Cool (2005) focused on the sesquiterpenes of *C. macrocarpa* and identified several new sesquiterpenes.

The volatile leaf oils of *Cupressus goveniana* appear to have only been analyzed by Zavarin et al. (1971) and that report was confined to the monoterpenes.

The monoterpenes of the volatile leaf oils of *Cupressus abramsiana* were reported by Zavarin et al. (1971). Jolad et al. (1984) reported the isolation of cupresol from *C. abramsiana*.

Cool et al. (1994) reported the occurrence of karahanaenone in trace or small amounts in *Cupressus abramsiana*, *C. forbesii*, *C. goveniana*, and *C. stephensonii*. However, they found individuals of *C. pygmaea* and *C. sargentii* whose oil contained over 20% concentrations of karahanaenone.

No analyses have been made of the volatile leaf oils of the new subspecies of *Cupressus* proposed by Silba (2003). Thus, we present below analyses of the leaf essential oils of *Hesperocyparis abramsiana* (C. B. Wolf) Bartel, *H. goveniana* (Gordon) Bartel, and *H. macrocarpa* (Hartw. ex Gordon) Bartel and compare these oils with the Silba's putative subspecies.

MATERIAL AND METHODS

Plant material - Specimens used in this study: *H. abramsiana*, Bonny Doon, Santa Cruz Co., CA, *Bartel 1598a-e*; *C. abramsiana* subsp. *butanoensis*, Pescadero Creek County Park, Butano Ridge, San Mateo Co., CA, *Bartel 1605a-e*; *C. abramsiana* subsp. *locatellii*, Eagle Rock, Santa Cruz Co., CA, *Bartel 1599a-e*; *C. abramsiana* subsp. *neolomondensis*, Wilder Ranch State Park, Santa Cruz Co., CA, *Bartel 1604a-e*; *C. a.* subsp. *opleri*, Bracken Brae, Santa Cruz Co., CA, *Bartel 1600a-e*; *H. goveniana*, SFB Morse Botanical Reserve, Monterey Co., CA, *Bartel 1596a-e*; *C. goveniana* subsp. *gibsonensis*, Point Lobos Ranch, Monterey Co., CA, *Bartel 1595a-e*; *H. pygmaea*, Albion Ridge, Mendocino Co., CA, *Bartel 1601a-e*; Little River Airport, *Bartel 1602a-e*; Casper Little Lake Rd., CA, *Bartel 1603a-e*; *C. macrocarpa* subsp. *lobosensis*, Point Lobos State Reserve, Allan Memorial Grove, Monterey Co., CA, *Bartel 1593a-e*, Point Lobos State Reserve, East Grove, *Bartel 1594a-e*; *H. macrocarpa*, Crocker Grove, Monterey Co.,

CA, Bartel 1597a-e. Voucher specimens currently are held in Bartel's personal herbarium in Carlsbad, California.

Isolation of Oils - Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

Chemical Analyses - Oils from 5-10 trees of each of the taxa were analyzed and both average and individual values are reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see 5 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2006), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

Data Analysis - Terpenoids (as per cent total oil) were coded and compared among the species by use of the Gower metric (1971). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

The leaf oils of *H. abramsiana* are dominated (table 1) by umbellone (16-21.4%), terpinen-4-ol (11.9 - 16.8%), and nezukol (6.1 - 12.1%) with moderate amounts of sabinene (7.5 - 9.6%), and β -phellandrene (7.3 - 9.4%). However, the *neolomondensis* population sample contained 3 individuals (neol, table 1) with high amounts of karahanaenone and α -terpinyl acetate as found in *H. pygmaea*. In fact, the oils of the *neolomondensis* - neol plants share two unique compounds with *H. pygmaea* (pyg, table 1): (Z)-nuciferol and β -(Z)-curcumen-12-ol as well as similar quantities of sabinene, camphor,

karahanaenone, terpinen-4-ol, 3-thujanol acetate, 4-terpinyl acetate, α -terpinyl acetate, and nezukol.

The leaf oils of *H. goveniana* were dominated by sabinene (15.2 - 26.3%), terpinen-4-ol (9.5 - 15.7%) and nezukol (11.1-26.3%) with moderate amounts of γ -terpinene (3.1-7.5%). *Hesperocyparis pygmaea* has also been treated as a subspecies of *H. goveniana*, but for this discussion it is treated as a species. The oil of *H. pygmaea* was not typical of *H. goveniana* in having a very high amount of karahanaenone (14.6%, table 1), camphor (8.7%), α -terpineol (3.2%) and α -terpinyl acetate (4.2%).

Table 1 shows that both *H. macrocarpa* oils are high in sabinene (27.0, 23.3%), α -pinene (22.2, 19.8%), terpinen-4-ol (11.7, 14.7%) with moderate amounts of γ -terpinene (5.6, 5.1%), isophyllocladene (4.4, 4.9%), myrcene (3.6, 3.2%), β -pinene (2.6, 2.0%) and phyllocladene (2.3, 2.0%). Of the 71 compounds identified, these subspecies seemed differ in only nezukol (0, 2.2%), citronellal (0.6, 0.3%) and piperitone (0, 0.3%). Clearly, the oils are nearly identical in both composition and component amounts (table 1).

To examine the overall similarities of the oils, a Principal Coordinates Ordination (PCO) was performed on the mean oils of the eleven taxa. Figure 1 shows the ordination based on 23 terpenoids (each greater than 1.0% of the oil). *Hesperocyparis pygmaea* is quite separated from *H. goveniana* in this PCO (Fig. 1). As mentioned above, three individuals of *neolomondensis* had oils that were high in karahanaenone and α -terpinyl acetate as found in *H. pygmaea*. The mean values of compounds are designated as AN1 in table 1 and figure 1. The mean values of the other two individuals (low in karahanaenone) are designated as AN2 in table 1 and figure 1. The oil of *neolomondensis*, type 1 (AN1) is most similar to *H. pygmaea* (Fig. 1), whereas *neolomondensis*, type 2 oil (AN2) is most similar to other *H. abramsiana* populations. Though not entirely unique, the Butano Ridge population grows on a sandstone outcrop surrounded by a dense canopy redwood forest. The oil of *butanoensis* (AB, Fig. 1) appears to be a little different from other *H. abramsiana* oils.

Hesperocyparis abramsiana appeared to show some infra-specific variation (Fig. 1, Table 1). A PCO analysis of individuals of *C.*

abramsiana from all five putative subspecies was made and is shown in Figure 2. The three individuals from *neolomondensis*, high in karahanaenone, group with *H. pygmaea*, whereas the other two plants of *neolomondensis* are imbedded with other *abramsiana* plants (Fig. 2). The individuals of *butanoensis* form a group somewhat distinct from other *abramsiana* individuals. The individuals of *H. abramsiana*, and putative subspecies *locatellii* and *opleri* are interspersed (Fig. 2). The PCO offers no support for the recognition of *locatellii* or *opleri*

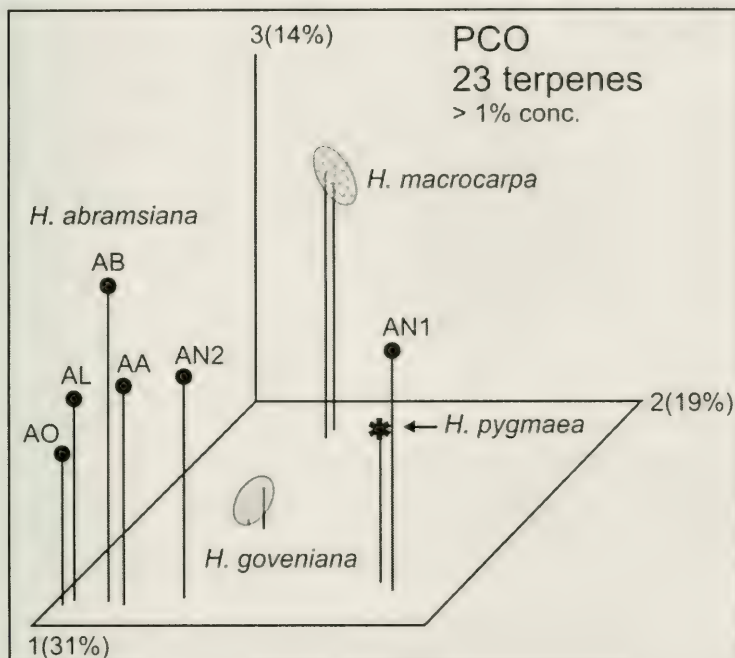


Figure 1. PCO of *Hesperocyparis* taxa using 23 terpenes that occurred in 1.0% or greater concentration.

AA = *H. abramsiana*, Bonny Doon

AB = *C. a. subsp. butanoensis*, Butano Ridge

AL = *C. a. subsp. locatellii*, Eagle Rock

AN1 = *C. a. subsp. neolomondensis*, high karahanaenone

AN2 = *C. a. subsp. neolomondensis*, low karahanaenone

AO = *C. a. subsp. opleri*, Bracken Brae

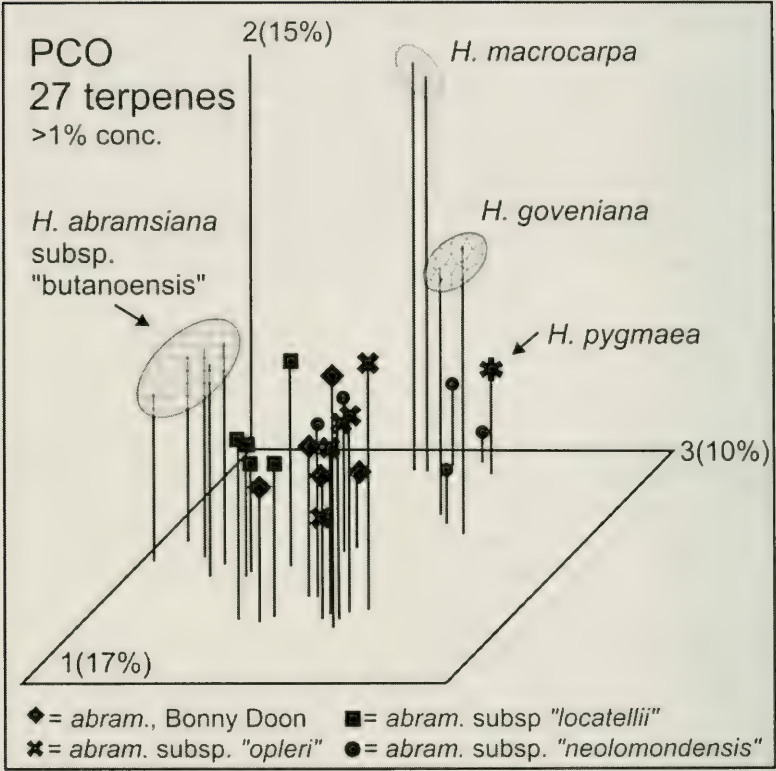


Figure 2. PCO of individuals of *H. abramsiana* based on 27 terpenes.

The initial analysis of *H. macrocarpa* subsp. *macrocarpa* and *C. m.* subsp. *lobosensis* average leaf oils (Fig. 1, Table 1) indicated that the oils were very similar with scarcely any differences (Table 1). PCO analysis of the individuals of *H. macrocarpa* confirm the overall trend. The individuals are interspersed (Fig. 3) implying that these two subspecies are behaving as one large population. We found no support in the leaf oil data to support the recognition of Silba's *C. abramsiana* subsp. *lobosensis*.

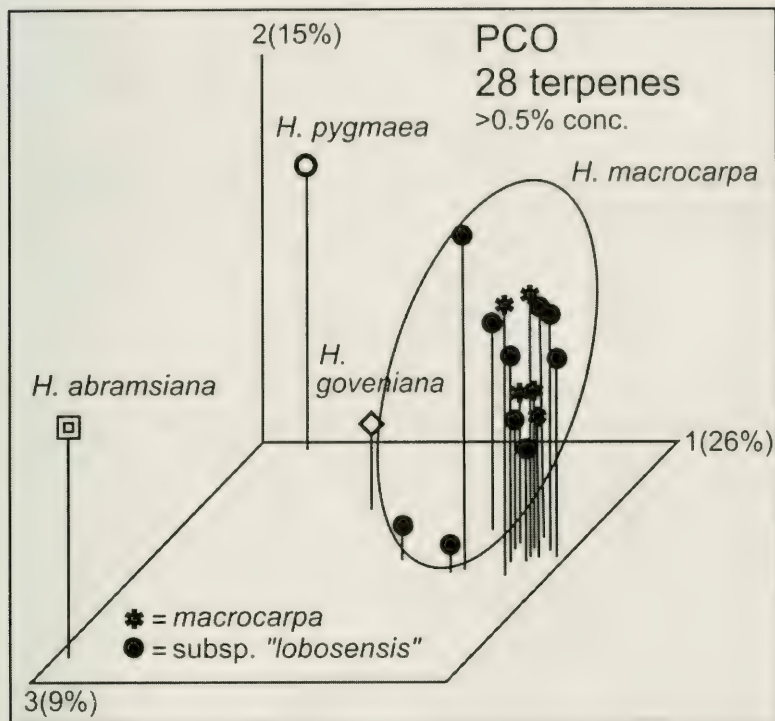


Figure 3. PCO of *H. macrocarpa* and Silba's putative *C. m.* subsp. *lobosensis* individuals along with mean values of *H. abramsiana*, *H. goveniana*, and *H. pygmaea*.

To examine differences among *H. goveniana*, putative *C. g.* subsp. *gibsonensis*, and *H. pygmaea*, a PCO analysis was made and is shown in figure 4. A slight separation exists between *H. pygmaea* and *H. goveniana* (Fig. 4), while there seems to be no difference between *H. goveniana* and Silba's putative *C. g.* subsp. *gibsonensis*, as these individuals are intermixed (Fig. 4). The three high karahanaenone individuals of *neolomondensis* were also included in the analysis and these seem close, but not conspecific with *H. pygmaea* (Fig. 4). Possibly these plants are the result of relictual or current hybridization

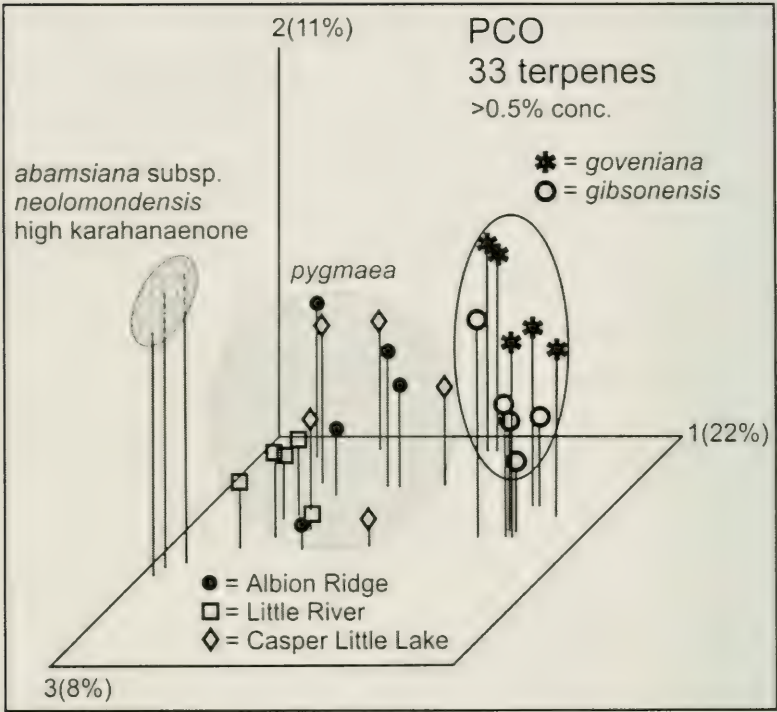


Figure 4. PCO of individuals of *H. goveniana*, putative *C. g. subsp. gibsonensis* and *H. pygmaea* plus three plants of putative *C. subsp. neolomondensis*, high karahanaenone type.

between *H. abramsiana* and *H. pygmaea*. Additional research using DNA markers is in progress to aid in resolving this situation. It should be noted that while each of the subspecies described by Silba (2003) are geographically isolated from one another, the individuals from the putative subspecies generally did not cluster geographically, but rather were randomly interspersed within each species as one would expect with an interbreeding population.

In summary, the leaf oils of putative *C. a. subsp. butanoensis*, Butano Ridge, showed some differentiation from *H. abramsiana*, Bonny Doon. The three individuals of putative *C. a. subsp.*

neolomondensis with high karahanaenone (and other components), seem intermediate to *H. pygmaea*. Additional research is needed to resolve this problem.

None of the new subspecies proposed by Silba (2003), (*C. abramsiana* subsp. *locatellii*, *C. a.* subsp. *opleri*, *C. goveniana* subsp. *gibsonensis*, *C. macrocarpa* subsp. *lobosensis*) is supported by differentiation of their volatile leaf oils, except possibly *C. a.* subsp. *butanoensis*.

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Table 1. Compositions of the leaf oils of *Hesperocyparis abramsiana* (abr), *C. a. subsp. locatellii* (loc), *C. a. subsp. opleri* (opl), *C. a. subsp. butanoensis* (but), *C. a. subsp. neolomondensis*, Type 2, low karahanaenone (neo2), *C. a. subsp. neolomondensis*, Type 1, high karahanaenone (neol), *H. pygmaea* (pyg), *H. goveniana* (gov), *C. g. subsp. gibsonensis* (gib), *H. macrocarpa* (mac), and *C. m. subsp. lHobosensis* (lob).

AI	compound	<i>H. abramsiana</i>									
		abr	loc	opl	but	neo2	neol	pyg	gov	gib	mac
846	(E)-2-hexenal	0.4	0.4	0.2	0.2	0.2	0.2	1.1	0.3	0.7	0.1
908	isobutyl-isobutyrate	-	-	-	-	0.1	0.4	-	0.3	-	-
909	2-methyl-propanic acid, butyl ester	t	t	0.2	-	-	-	-	-	-	-
921	tricyclene	0.2	t	t	0.1	0.1	0.3	0.1	t	0.1	0.1
924	α -thujene	1.0	1.9	0.8	2.0	0.8	0.3	0.6	0.9	0.7	1.2
932	α -pinene	1.3	3.5	1.2	10.5	2.9	2.3	3.0	1.6	1.6	22.2
945	α -fenchene	-	-	-	-	-	-	0.1	-	-	t
946	camphene	0.4	t	t	0.2	0.3	0.6	0.3	0.2	0.2	0.4
969	sabinene	7.5	9.6	7.1	11.6	8.6	7.4	15.2	26.3	19.4	27.0
974	β -pinene	0.1	0.2	0.1	0.8	0.3	0.1	0.3	0.3	1.0	2.6
988	myrcene	2.5	3.9	3.7	4.1	2.3	3.2	2.6	3.7	2.9	3.6
1002	α -phellandrene	0.3	0.4	0.6	0.4	0.4	0.8	0.6	0.4	0.4	0.2
1008	δ -3-carene	t	0.1	t	0.2	-	-	1.3	6.4	t	0.4
1014	α -terpinene	2.7	3.6	3.8	3.0	2.5	1.6	1.9	4.2	3.7	3.2
1020	p-cymene	0.9	1.0	1.3	1.4	0.8	0.3	0.5	0.5	0.3	0.3
1024	limonene	3.0	4.0	3.0	7.3	2.1	2.2	1.2	1.8	1.5	0.8

Al	compound	<i>H. abramsiana</i>					<i>H. pyg H. goveniana H. macrocarpa</i>					
		abr	loc	opl	but	neo2	neol	pyg	gov	gib	mac	lob
1025	β-phellandrene	7.8	9.4	7.3	4.0	4.9	5.2	1.5	1.8	1.5	1.1	1.0
1026	1,8-cineole	-	-	-	-	-	t	0.1	-	1.2	-	-
1044	(E)-β-ocimene	t	t	0.1	0.1	0.1	0.1	0.1	-	-	-	t
1054	γ-terpinene	3.6	4.2	5.2	4.2	3.7	2.4	3.1	7.5	6.2	5.6	5.0
1065	cis-sabinene hydrate	0.6	0.5	0.5	0.4	0.2	0.1	0.5	0.8	0.5	1.0	1.4
1073	2-methyl nonanone*	0.2	-	0.5	0.1	0.3	1.4	0.3	0.2	0.2	-	-
1086	terpinolene	2.2	3.2	2.6	2.7	1.7	1.0	1.3	2.9	2.0	2.1	1.8
1088	isobutyl tiglate	0.1	-	0.1	0.1	0.1	0.6	-	-	-	-	-
1090	6,7-epoxymyrcene	-	-	-	-	-	-	-	-	-	0.2	0.1
1096	trans-sabinene hydrate	0.4	0.7	t	0.4	0.1	t	0.3	0.6	0.5	1.4	1.5
1096	linalool	1.0	t	3.1	0.1	0.1	0.9	0.7	0.6	0.1	-	-
1100	n-nonanal	t	-	t	0.1	0.1	-	0.1	0.1	0.2	t	t
1112	trans-thujone	-	-	-	0.1	0.1	0.1	0.1	0.1	0.1	-	-
1118	cis-p-menth-2-en-1-ol	0.9	0.8	1.1	0.6	0.5	0.4	0.5	0.9	0.7	0.7	0.8
1122	α-campholenal	-	-	-	-	-	-	-	-	-	0.1	0.2
1136	trans-p-menth-2-en-1-ol	0.4	0.6	0.8	0.5	0.4	0.2	0.3	0.9	0.5	0.6	0.7
1137	trans-verbenone	-	-	-	-	-	-	-	-	-	t	0.2
1141	camphor	12.4	0.1	1.5	0.6	6.5	13.5	8.7	0.2	1.8	0.3	0.1
1145	camphene hydrate	0.5	t	0.2	t	0.3	0.5	0.4	-	0.2	0.1	0.2
1154	sabina ketone	-	-	-	-	t	-	-	-	-	-	-

Al	compound	<i>H. abramsiana</i>					<i>H. pyg</i> <i>H. goveniana</i> <i>H. macrocarpa</i>					
		abr	loc	opl	but	neo2	neol	pyg	gov	gib	mac	lob
1148	citronellal	0.2	t	0.2	-	-	-	t	0.5	-	0.6	0.2
1152	(3Z)-nonen-1-ol	-	-	-	-	-	-	-	-	-	t	t
1154	karahanaenone	-	-	-	-	-	18.4	14.6	2.2	0.9	-	-
1165	borneol	-	-	-	-	-	-	t	-	0.8	0.1	0.3
1167	umbellulone	21.4	20.8	16.0	21.1	10.7	0.9	-	-	-	-	-
1174	terpinen-4-ol	12.8	11.9	16.8	9.5	9.7	6.6	9.5	15.7	13.9	11.7	14.5
1179	p-cymen-8-ol	-	-	-	0.2	0.3	t	t	0.3	t	t	0.2
1183	cryptone	0.2	0.4	0.9	0.1	-	-	-	-	-	-	-
1186	α -terpineol	1.2	0.7	1.9	0.8	0.7	1.6	3.2	0.9	0.6	0.8	0.8
1195	cis-piperitol	0.3	0.2	0.3	0.2	0.1	t	t	0.2	0.2	0.2	0.2
1198	shisofuran	0.2	0.1	0.1	0.2	0.1	-	-	-	-	-	-
1207	trans-piperitol	0.3	0.3	0.4	0.2	0.2	0.1	0.2	0.4	0.3	0.4	0.4
1223	citronellol	1.3	0.3	0.6	0.2	0.2	1.2	2.2	1.1	2.2	1.1	0.8
1232	thymol, methyl ether	0.1	0.2	0.1	0.1	0.1	-	-	-	-	-	-
1241	carvacrol, methyl ether	-	-	-	0.2	-	-	t	-	-	0.1	0.3
1249	piperitone	0.3	0.2	0.3	0.2	0.2	t	0.1	0.1	-	-	0.2
1287	bornyl acetate	0.3	-	-	0.1	0.3	0.9	-	-	0.2	-	-
1289	thymol	0.1	0.1	0.1	0.1	-	-	-	-	-	-	-
1293	2-undecanone	-	-	-	-	-	-	t	0.3	0.2	-	-
1295	3-thujanol acetate	-	-	-	0.2	0.2	1.0	0.1	-	-	-	-

AI	compound	<i>H. abramsiana</i>				<i>H. pyg</i>				<i>H. goveniana</i>				<i>H. macrocarpa</i>	
		abr	loc	opl	but	neo2	neol	pyg	gov	gib	mac	lob			
1298	carvacrol	-	-	-	-	-	t	-	-	-	t	t			
1299	4-terpinyl acetate	0.2	0.3	0.6	-	-	0.2	0.1	-	-	-	-			
1346	α -terpinyl acetate	0.3	0.1	0.2	0.2	0.2	7.1	4.2	0.1	-	-	t			
1350	citronellyl acetate	0.1	-	-	t	-	0.2	t	-	t	-	-			
1390	unknown, <u>43</u> , 118, 107, 210	0.8	0.4	0.2	-	-	-	-	-	-	-	-			
1403	methyl eugenol	-	-	-	-	-	t	t	0.3	0.2	0.1	t			
1452	α -humulene	-	-	-	0.1	-	-	-	-	-	-	-			
1470	α -macrocarpene	-	-	-	-	-	-	-	-	-	0.1	0.1			
1480	germacrene D	-	-	-	-	-	-	t	-	-	-	-			
1499	β -macrocarpene	-	-	-	-	-	-	-	-	-	t	t			
1505	β -bisabolene	0.1	t	0.2	-	-	t	t	-	-	-	-			
1508	germacrene A	-	-	-	-	-	-	-	-	-	t	t			
1522	δ -cadinene	-	-	-	-	-	-	-	-	-	t	t			
1548	elemol	-	-	-	-	-	-	-	0.2	0.2	0.1	0.2			
1559	germacrene B	-	-	-	-	t	t	t	0.3	0.2	0.1	0.1			
1561	(E)-nerolidol	0.1	t	t	0.1	t	t	t	-	-	0.1	0.1			
1574	germacrene D-4-ol	-	-	-	-	-	-	-	-	-	t	t			
1600	cedrol	-	-	-	-	-	-	-	0.1	0.2	-	-			
1630	γ -eudesmol	-	-	-	-	-	-	-	0.1	t	t	t			
1638	epi- α -cadinol	-	-	-	-	-	-	-	-	-	t	t			
1638	epi- α -muurolol	-	-	-	-	-	-	-	-	t	t	t			

		<i>H. abramsiana</i>				<i>H. pyg</i> <i>H. goveniana</i> <i>H. macrocarpa</i>						
Al	compound	abr	loc	opl	but	neo2	neol	pyg	gov	gib	mac	lob
1649	β -eudesmol	-	-	-	-	-	-	-	t	t	t	t
1652	α -eudesmol	-	-	-	-	-	-	-	t	t	t	0.1
1652	α -cadinol	-	-	-	-	-	-	-	t	t	t	0.1
1683	epi- α -bisabolol	0.2	t	0.5	t	t	0.3	t	0.1	0.1	-	-
1715	(2Z,6E)-farnesol	-	-	-	-	-	-	-	t	t	t	0.3
1724	(Z)-nuciferol	-	-	-	-	-	t	t	-	-	-	-
1754	β -(Z)-curcumen-12-ol	-	-	-	-	-	t	t	-	-	-	-
1896	rimuene	t	t	t	t	0.3	0.1	t	t	t	-	-
1900	pimara-9(11),15-diene*	0.1	0.2	0.2	0.2	0.9	0.3	1.1	0.2	0.5	0.1	0.1
1905	isopimara-9(11),15-diene	0.1	0.2	0.3	t	0.6	0.1	0.2	t	0.1	t	t
1925	diterpene, <u>41</u> , 106, 257, 272	0.1	0.2	t	0.2	t	0.3	t	-	-	0.8	0.9
1948	pimaradiene	t	0.2	-	0.1	0.2	0.1	t	-	-	-	0.1
1960	iso-sandaracopimara-8(14),15-diene	0.2	0.7	0.3	t	t	t	t	0.2	0.4	-	-
1966	isophyllocladene	0.5	1.1	0.5	1.8	0.8	1.8	0.4	-	-	4.4	4.0
1978	manoyl oxide	0.7	0.3	1.3	0.1	2.3	1.2	0.5	0.8	2.2	t	-
2009	epi-13-manoyl oxide	0.1	0.1	0.2	-	0.4	0.2	0.1	t	-	-	-
2016	phyllocladene	0.1	0.1	0.1	0.2	-	0.2	0.1	t	0.5	0.6	0.6

AI	compound	<i>H. abramsiana</i>					<i>H. pyg</i> <i>H. goveniana</i> <i>H. macrocarpa</i>					
		abr	loc	opl	but	neo2	neol	pyg	gov	gib	mac	lob
2055	abietatriene	t	0.1	t	0.2	0.2	0.1	0.1	0.1	t	0.2	0.1
2091	iso-nezukol*	0.2	0.2	0.3	0.2	0.7	0.2	0.2	0.1	0.4	-	-
2105	iso-abienol	-	-	-	-	-	t	t	0.1	t	-	-
2132	nezukol	6.1	10.4	12.1	6.5	29.1	10.8	13.2	11.1	26.3	t	2.2
2184	sandaracopimarinal	0.1	0.2	0.1	0.2	0.2	0.1	0.1	t	0.1	t	t
2209	phyllocladanol	-	-	-	-	-	-	-	-	-	2.3	1.9
2269	sandaracopimarinal	t	0.2	t	-	-	-	-	t	-	-	t
2282	sempervirol	0.1	0.3	0.3	0.2	0.5	0.2	0.3	0.6	0.4	0.2	0.4
2314	trans-totarol	t	0.3	0.1	0.4	0.4	0.3	t	0.4	0.3	0.2	0.3
2331	trans-ferruginol	t	0.2	t	0.1	0.1	0.1	t	2.0	t	0.1	0.2

AI = Arithmetic Index on DB-5 column. Values less than 0.05% are denoted as traces (t). Unidentified components less than 0.5% are not reported. Those compounds that appear to distinguish taxa are in boldface.

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**GEOGRAPHIC VARIATION IN *HESPEROCYPARIS*
(*CUPRESSUS*) *ARIZONICA* AND *H. GLABRA*:
RAPDS ANALYSIS**

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ABSTRACT

RAPDs were analyzed from five *Hesperocyparis* (= *Cupressus*) *arizonica* and three *H. glabra* populations. This analysis supports the continued recognition of these taxa at the specific level. *Phytologia* 91(2): 244-250 (August, 2009).

KEY WORDS: *Hesperocyparis* (= *Cupressus*) *arizonica*, *H. glabra*, RAPDs, geographic variation, taxonomy.

Hesperocyparis (= *Cupressus*) *arizonica* (Greene) Bartel and *H. glabra* (Sudw.) Bartel are two closely related taxa that have a variable taxonomic history. Table 1 summarizes the taxonomic treatments. Wolf (1948) recognized both taxa at the specific level (Table 1), while Little (1970) reduced *C. glabra* to a variety (*C. arizonica* var. *glabra*). Though Bartel (1993) and Eckenwalder (1993) included *C. glabra* within *C. arizonica*, Farjon (1998) followed Little (1970) in recognizing *C. glabra* as a variety of *C. arizonica* (Table 1). All these classifications were based strictly on morphology.

Askew and Schoenike (1982) concluded that bark texture (fibrous and not peeling = *H. arizonica* versus smooth and peeling in thin plates or strips = *H. glabra*) correctly identified the taxa 89% of the time, while resin gland occurrence worked 85% of the time. However, Little

(2006) separated these taxa using only resin glands (on < 5% of leaves =*H. arizonica* versus on >5% of leaves =*H. glabra*) in his key..

Table 1. Taxonomic treatments of *H. arizonica* and *H. glabra*.

Treatment	<i>arizonica</i>	<i>glabra</i>
Wolf (1948)	<i>C. arizonica</i>	<i>C. glabra</i> .
Little (1970)	<i>C. arizonica</i> var. <i>arizonica</i>	<i>C. arizonica</i> var. <i>glabra</i> (Sudw.) Little (= <i>C. arizonica</i>)
Bartel (1993)	<i>C. arizonica</i>	(= <i>C. arizonica</i>)
Eckenwalder (1993)	<i>C. arizonica</i>	(= <i>C. arizonica</i>)
Farjon (1998)	<i>C. arizonica</i> var. <i>arizonica</i>	<i>C. arizonica</i> var. <i>glabra</i> (Sudw.)
Bartel et al. (2003)	<i>C. arizonica</i>	<i>C. glabra</i> Sudw.
D. P. Little (2006)	<i>Callitropsis arizonica</i> (Greene) D. P. Little	<i>Callitropsis. glabra</i> (Sudw.) D. P. Little
Adams et al.(2009)	<i>Hesperocyparis arizonica</i> (Greene) Bartel	<i>Hesperocyparis glabra</i> (Sudw.) Bartel

A recent analyses using RAPDs fingerprinting (Bartel et al., 2003) found *H. glabra* to be distinct from *H. arizonica* (Fig. 1).

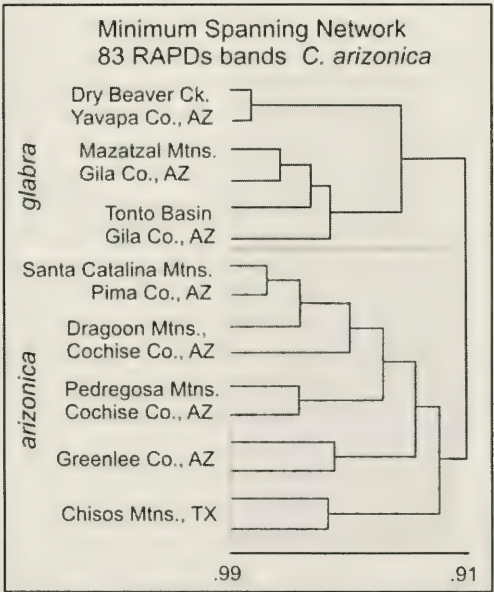


Figure 1. Minimum spanning network (from Bartel et al., 2003).

Recent DNA sequencing of *Cupressus sensu lato* (Little et al., 2004, Little, 2006) demonstrated that the Western Hemisphere species form a well-supported clade quite separated from the Eastern Hemisphere cypresses. As a result, Little (2006) not only confined the genus *Cupressus* to the Eastern Hemisphere, he used *Callitropsis nootkatensis* and its generic epithet for the Western Hemisphere cypresses and *Xanthocyparis vietnamensis*.

Little (2006) found very limited nucleotide differences among any of the Western Hemisphere *Hesperocyparis* species. However, he did find differences that supported the recognition of *H. arizonica* and *glabra* (Table 2) and he maintained these two taxa as distinct species.

Table 2. Summary of DNA sequencing support for the recognition of *H. arizonica* and *H. glabra* (from Little, 2006).

Chloroplast genes	Nuclear genes	
<i>matK+rbcL+trnL</i>	nrDNA(ITS)	<i>NEEDLY</i>
60% support	56% support	no differences

Debreczy et al. (2009) argued on morphological grounds that *Ca. nootkatensis* is a monotypic genus. Sequencing by Adams et al. (2009) of two additional nuclear genes and petN-psbM further supported the recognition of *Ca. nootkatensis* as a monotypic genus. Because *Callitropsis* should not be applied to the Western Hemisphere cypresses, Bartel and Price in Adams et al. (2009) described a new genus, *Hesperocyparis*, for the Western Hemisphere cypresses (exclusive of *Xanthocyparis vietnamensis* and *Callitropsis nootkatensis*). In Adams et al. (2009), Bartel made the new combinations of *Hesperocyparis arizonica* (Greene) Bartel and *H. glabra* (Sudw.) Bartel. The present paper will analyze geographical variation within and between *H. arizonica* and *H. glabra*.

MATERIALS AND METHODS

Collection information for specimens utilized:

Hesperocyparis arizonica: Adams 9268-9269, Boot Spring, Chisos Mtns., Brewster Co., TX, USA; Lab # 9378-9379,

Bartel, 1580A,B, upper Bear Canyon, 11.8 mi n of Houghton Rd along Catalina Hwy, N 32 21' 47.9", W 110 42' 50.3", 1695m, Santa Catalina Mtns., Pima Co., AZ; Lab # 9380-9381, Bartel, 1581A,B, Stronghold Canyon East, 7.3 mi from US 191, along Ironwood Rd., N 31 56 26.9", W 109 57' 27.8" 1457m, Dragoon Mtns., Cochise Co., AZ; Lab # 9382-9383, Bartel, 1582A, B, Rucker Canyon, 6.1 mi from Leslie Canyon Rd along Rucker Canyon Rd., N 31 45' 18.3", W 109 22' 39.5", 1676m, Pedregosa Mtns., Cochise Co., AZ, 9384-9385, Bartel, 1583A,B, Metcalf, w of Chase Creek, 9.6 mi from lower Eagle Creek Rd, along US191, N 33 08' 01.5", W 109 22' 38.7", 1683m, Greenlee Co., AZ,

Hesperocyparis glabra, 9386-9387, Bartel, 1584A,B, upper Slate Creek, 7.1 mi sw of SR 188, along SR87, N 33 57' 28.5", W 111 24' 21.8", 1009m, Mazatzal Mtns., Gila Co., AZ, 9388-9389, Bartel, 1585A,B, se of Tonto Natural Bridge St. Park, jct along SR87, nw of East Verde River, N 34 18' 58.6", W 111 23' 12.6", 1483m, Gila Co., AZ, 9390-9391, Bartel, 1586A,B, upper Dry Beaver Creek, 0.1 mi. e of SR 179 along Wild Horse Mesa Rd., N 34 46' 07.7", W 111 45' 46.4", 1225m, Yavapai Co., AZ.

Adams' specimens are deposited at BAYL herbarium, Waco, Texas. Bartel's specimens currently are held in his personal herbarium, Carlsbad, California.

One gram (fresh weight) of foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20 °C until the DNA was extracted. DNA was extracted from the leaves by use of the Qiagen DNeasy Mini-plant extractors. Ten-mer primers were purchased from the University of British Columbia (5'-3'): 131, GAA ACA GCG T; 153, GAG TCA CGA G; 204, TTC GGG CCG T; 212, GCT GCG TGA C; 218, CTC AGC CCA G; 239, CTG AAG CGG A; 244, CAG CGA ACC G; 250, CGA CAG TCC C; 327, ATA CGG CGT C; 338 CTC TGG CGG T; 346, TAG GCG AAC G; 347, TTG CTT GGC G; 389 CGC CCG CAG T; 413, GAG GCG GCG A;

PCR was performed in a volume of 15 ml containing 50 mM Tris-HCl (pH 9), 2.0 mM MgCl₂, 0.01% gelatin and 0.1% Triton X-100, 0.2 mM of each dNTPs, 0.36 mM primers, 0.3 ng genomic DNA, 15 ng BSA and 0.6 unit of Taq DNA polymerase (Promega). A control

PCR tube containing all components, but no genomic DNA, was run with each primer to check for contamination. DNA amplification was performed in an MJ Programmable Thermal Cycler (MJ Research, Inc.). The thermal cycle was: 94 C (1.5 min) for initial strand separation, then 40 cycles of 38 C (2 min), 72 C (2 min), 91 C (1 min). Two additional steps were used: 38 C (2 min) and 72 C (5 min) for final extension. Bands that occurred once or did not show fidelity within the two replicated samples of each taxon were eliminated. It should be noted that these bands contain very useful information for the study of genetic variance and individual variation, but are merely "noise" in the present taxonomic study. Bands were scored in 4 classes: very bright (=6); medium bright (=5), faint (=4) and absent (=0). See Adams and Demeke (1993) for details on electrophoresis and RAPD band scoring.

Similarity measures were computed using absolute character state differences (Manhattan metric), divided by the maximum observed value for that character over all taxa (= Gower metric, Gower 1971; Adams 1975). Principal coordinate analysis (PCO) of the similarity matrix follows Gower (1966).

RESULTS AND DISCUSSION

Contoured clustering based on 83 RAPD bands (Figure 2) shows that the populations cluster by geographical proximity. The most similar *H. arizonica* populations are Santa Catalina Mtns. - Dragoon Mtns. (0.969), followed by Dragoon Mtns. - Pedregosa Mtns. (0.943), then Pedregosa Mtns. - Greenlee Co. (0.932). The Chisos Mtns., TX population is clearly quite differentiated (linkage of 0.926 to the Dragoon Mtns. population).

The most similar *H. glabra* populations are Mazatzal Mtns. - Tonto Basin (0.959), then Tonto Basin - Dry Beaver Creek (0.937). The *H. arizonica* - *H. glabra* populations are finally linked by Greenlee Co. - Mazatzal Mtns. (0.916).

The linkage of populations of both taxa by geographically near neighbors suggests that differentiation is due to restricted gene exchange perhaps leading to genetic drift. Due to the likely short distances of cone/ seed dispersal, it seems probable that pollen dispersal

over long distances may be the principal agent of gene flow among these populations.

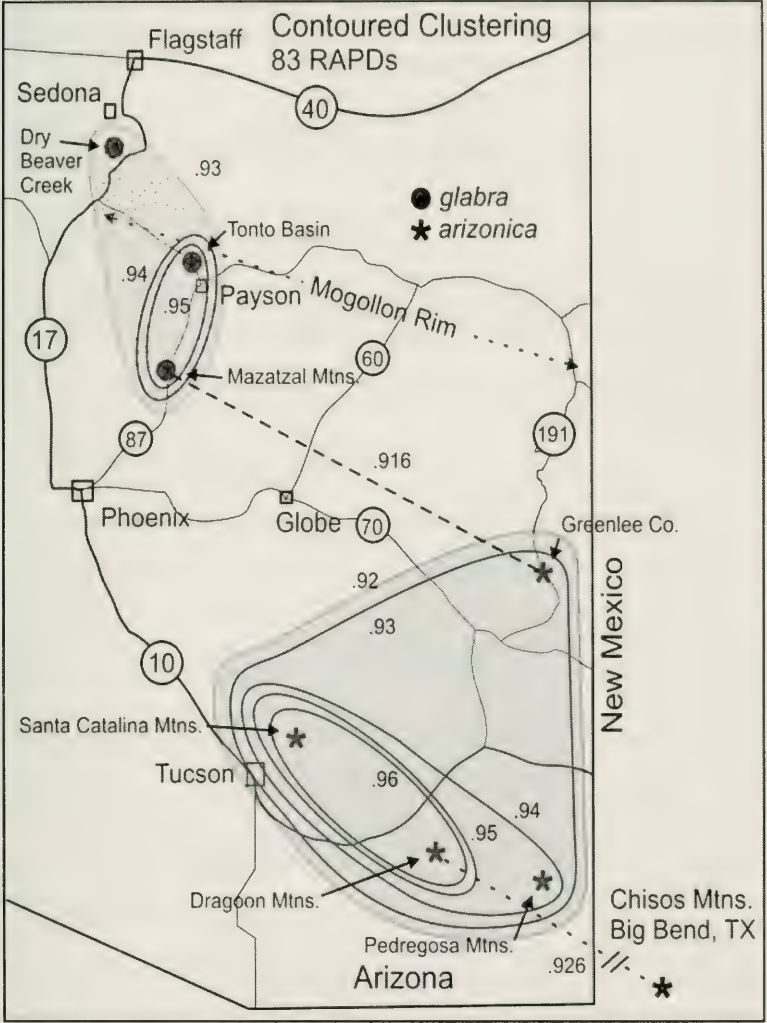


Figure 2. Contoured clustering of populations of *H. arizonica* and *H. glabra* based on 83 RAPDs bands.

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**SOLIDAGO DISPERSA (ASTERACEAE: ASTEREAE)
REPLACES SOLIDAGO LUDOVICIANA
AS THE CORRECT NAME**

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ABSTRACT

Solidago dispersa Small is the correct name for the species previously identified as *S. ludoviciana* (A. Gray) Small. The species is restricted to east Texas, western Louisiana, southern Arkansas, and the southeastern corner of Oklahoma. A distribution map is provided for *S. dispersa* and taxonomic with nomenclatural summaries for *S. dispersa* and its synonyms, *S. ludoviciana* and *S. strigosa* Small. *Phytologia* 91(2): 251-255 (August, 2009).

KEY WORDS: *Solidago ludoviciana*, *S. dispersa*, Asteraceae.

The correct name for the species generally identified as *Solidago ludoviciana* (A. Gray) Small is *S. dispersa* Small, as interpreted here. The distribution of *S. dispersa* is restricted to east Texas, western Louisiana, southern Arkansas, and the southeastern corner of Oklahoma (Fig. 1). In Oklahoma it is known from only two collections. **McCurtain Co.:** disturbed roadside with windrows of bulldozed trees, 2 mi S and 0.4 mi W of Tom along Hwy 87, 18 Sep 1976, *Taylor & Taylor* 23641 (BRIT, OKL); sloughy bank of Cedar Creek, 10 mi N of Broken Bow, 16 Oct 1937, *Hopkins & Cross* 2496 (OKL).

In resolving the nomenclature, observations on the morphology of *Solidago dispersa* are critical. Compared to *S. arguta* var. *boottii*, with which it is sometimes confused, the slender, scale-leaved, stoloniform rhizomes of *S. dispersa* are diagnostic. The two are sympatric in an area of northcentral Louisiana and adjacent Arkansas. The map in Fig. 1 is unambiguous and the coherence of the geographic

distribution supports the recognition of *S. dispersa*. The leaves of *S. dispersa* are thicker with denser reticulation (smaller interstices) of tertiary venation, and distal cauline leaves are erect-ascending and basally attenuate (vs. spreading and usually with an abruptly delimited petiole-like base in var. *boottii*). Plants of *S. dispersa* in Texas (except for some counties bordering Louisiana) are completely glabrous below the capitulescence. In Louisiana and Arkansas scattered plants are similarly glabrous but more commonly the lamina of basal and proximal cauline leaves are abaxially strigose-hirsute to hirsute; sometimes both faces have hairy lamina. The proximal and mid-region stems of such hairy-leaved plants commonly are sparsely to moderately hirsute. Plants of *S. arguta* var. *boottii* very rarely have leaves with lamina hairy abaxially (never adaxially) and none has been observed with hairy stems. Morton (1973) also recognized that *S. dispersa* is variable in vestiture.

As noted by Semple (2006, p. 132), "G.H. Morton annotated the type of [*Solidago dispersa*] as possibly being *S. arguta* introgressed with *S. ulmifolia*. The application of the name remains uncertain." The type collection of *S. dispersa*, however, was made in western Louisiana (Sabine Parish, on the Texas border), outside of the range of *S. arguta* but within that of *S. ludoviciana* and in an area where the latter is common.

The roots and lower stem of the type of *Solidago dispersa* were not collected and (fide Small's protologue, p. 476) "The inflorescence is paniculate and very loosely disposed [hence, apparently, the epithet], while the branches of the panicle and the elongated peduncles are filiform or nearly so and quite weak." Small also noted that "It is peculiar in being glabrous or nearly so above and with more or less pubescence on the lower leaves." The vestiture is similar to that of many plants from Louisiana previously identified as *S. ludoviciana*. The inflorescence is uncharacteristic, but plants of a few other collections otherwise typical in morphology for *S. ludoviciana* have elongated peduncles and heads in a 'loosely disposed' inflorescence, appearing more diffusely paniculate than short-pedunculate and strongly secund on spreading branches (e.g., Texas: Wood Co., *Lundell & Lundell 11741* (SMU); Bowie Co., *Correll & Correll 24789* (LL, SMU). These variants are very similar to the type

of *S. dispersa* and the type is thus within both the geographic and morphological bounds of the species. There is no apparent evidence that *Solidago dispersa* hybridizes with *S. ulmifolia*, *S. arguta*, or any other species. *Solidago dispersa* was observed by Cronquist (1980, as *S. ludoviciana*) to be closely similar to *S. arguta* and *S. tarda*. Fernald (1936) used the name *S. ludoviciana* to identify plants now known as *S. tarda* on the Atlantic coastal plain. Taylor and Taylor (1984) identified *S. dispersa* in Texas and Louisiana as *S. salicina* Ell., which in their concept also included *S. patula*.

Solidago dispersa Small, Bull. Torrey Bot. Club 25: 475. 1898. TYPE: U.S.A. Louisiana. No other data, but probably Sabine Parish, 1836–1838, *Leavenworth s.n.* (holotype: NY, internet image). Melines Conkling Leavenworth, an army physician, was stationed in Sabine Parish, Louisiana, in an active phase of his botanical work (1836–1838), during which he corresponded with John Torrey (ca. 25 letters) and sent him many specimens (McVaugh 1947). The type of *S. dispersa* probably was among these collections. Torrey and Gray (1842) cited collections by Leavenworth (and by Josiah Hale, see comments in typification of *S. strigosa* and *S. ludoviciana*) from Louisiana under "var. β " and "var. ϵ " of *S. boottii* (see comments under *S. strigosa*).

Solidago ludoviciana (A. Gray) Small, Fl. S.E. US. 1198, 1339. 1903. *Solidago boottii* var. *ludoviciana* A. Gray, Proc. Amer. Acad. Arts 17:195. 1882. LECTOTYPE (J.K. Small ex M.L. Fernald 1936, p. 210): U.S.A. Louisiana. No other data, *J. Hale s.n.* (NY 00259711, internet image!). No indication of a type was given in the protologue but Gray (1884, p. 154) noted "W. Louisiana." Most of Hale's Louisiana collections, which began in 1838, were made from the vicinity of Alexandria (Rapides Parish) and sent to Torrey and Gray (Ewan 2005). "Hale's plants were not numbered, and so after Charles Short and others had divided the original specimens and exchanged a portion, the origin ('Louisiana') was often all that accompanied the specimen" (Ewan 2005, p. 2282). Comments following *Solidago strigosa*, a name closely associated with *S. ludoviciana*, explain the lectotypification.

Solidago strigosa Small, Fl. S.E. US. 1198, 1339. 1903. *Solidago arguta* var. *strigosa* (Small) Steyererm., Rhodora 62:131. 1960.

TYPE: U.S.A. Louisiana. No other data, *J. Hale s.n.* (holotype: NY 00259966, internet image; isotype: GH 12437). Three Hale specimens of *Solidago* aff. *arguta* are housed at GH and NY: GH 12437; NY ...711 (identified on the original label as "*S. boottii* ϵ ?") and NY ...966 (originally identified as "*S. boottii* between β and ϵ "), each with an original "Torr. & Gray, Flora N. Amer." label. Gray studied the collections at both herbaria; he observed that NY ...711 was different from the GH collection, noting at the bottom of NY ...711 "My specimen of this is hirsute" and on the GH sheet "The specimen in Hb. Torr. of var. ϵ ? is glabrous." Gray (1884, p. 154) included both specimens in his concept of *S. ludoviciana*, describing the species as having "lower leaves and lower part of the stems sometimes roughish-hirsute or hispidulous with many-jointed hairs, **or glabrous**" [emphasis added].

Small apparently decided that NY ...711 was representative of *Solidago ludoviciana* and annotated it as such. Seeing that NY ...966, was different from ...711, he described ...966 as *S. strigosa*. Small's morphological and geographical descriptions of the two taxa (1903, 1933) support this interpretation. Fernald (1936) provided an account similar to the one here of the three Hale specimens; he also noted that "Small having selected the glabrous plant of Hale to stand as the type of *S. ludoviciana*, that point is satisfactorily settled, the hirsute plant of Hale being *S. strigosa* Small." Fernald's affirmation of Small's choice is taken here as formalization of the lectotypification of *S. ludoviciana*. Morton (1973) also concluded that the types of *S. strigosa* and *S. ludoviciana* are conspecific, despite the difference in vestiture.

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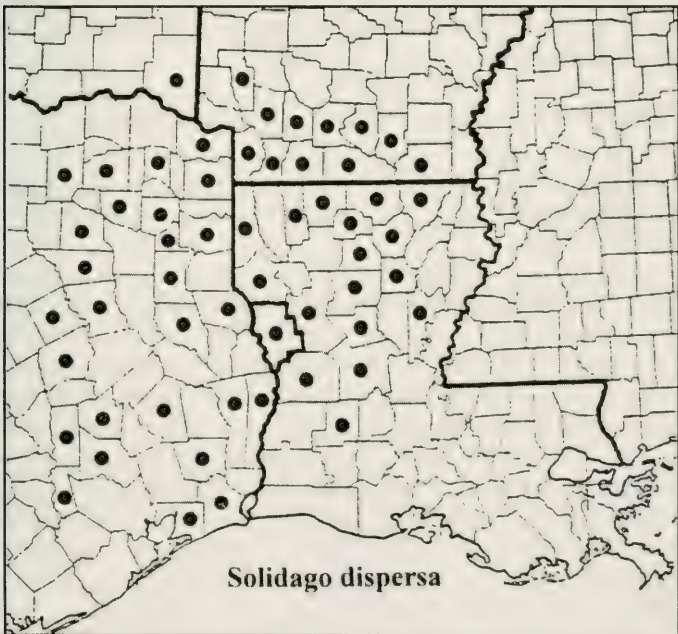


Figure. 1. Distribution of *Solidago dispersa*. Sabine Parish, Louisiana, where the type was collected, is outlined in bold.

RECENSION OF THE MEXICAN SPECIES OF *SALVIA* (LAMIACEAE), SECTION *SCORODONIA*

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ABSTRACT

A recension of the Mexican species of *Salvia* belonging to the sect. *Scorodonia* is rendered. Fourteen species are recognized, two of these described as new: *S. periconia*, a white-flowered taxon, from Oaxaca, and *S. tenorioi*, a yellow-flowered taxon from Puebla. A key to the various taxa is provided as well as photographs of the holotypes. Maps showing distribution of the taxa are provided. *Phytologia* 91(2): 256-269 (August, 2009).

KEY WORDS: *Salvia*, Lamiaceae, sect. *Scorodonia*, Mexico

43. Sect. *Scorodonia*

Sect. *Atratae* Epling

Perennial herbs, shrubs or subshrubs, mostly 1-5 m high. Leaves ovate to deltoid, rarely cordate; petioles mostly short with a well-defined abscission line near the base, rarely not; blades often bullate, variously pubescent, the margins crenate. Spikes various, usually terminal, interrupted or not, rarely spicate-paniculate. Floral bracts lanceolate to broadly ovate, early deciduous. Calyx with upper lip 5-7 veined. Corollas mostly purple to blue, sometimes white, rarely yellow. Stamens arising from near corolla throat, the anthers not exerted. Style branches pilose, the upper lobe 2-3 times as long as lower. Nutlets glabrous.

Type species: *S. melissodora* Lag.

Epling (1939) notes that sect. *Scorodonia* is "A group of closely related forms difficult to distinguish save on the summation of

minutiae in habit, yet the range both in structure and in distribution is such as to support the [treatment rendered].” So introduced, he recognized 11 species as occurring in Mexico. Ramamoorthy (1984) added to the assemblage *S. boegeri*; this, along with the two species described below bring to 14 the number of Mexican taxa currently recognized in the section.

Epling (1941) subsequently combined sect. *Urica* with sect. *Scorodonia*, but I have opted to retain the two, mainly because of the lack of a clearly defined abscission line at the base of the petiole in species of the sect. *Urica*.

Key to Mexican species

- 1. Corollas yellow; Hid.....**S. tenorioi**
- 1. Corollas not yellow...(2)
- 2. Corollas variously blue to purple...(4)
- 2. Corollas white or creamy white...(3)
- 3. Petioles 2-3 cm long; Sierra Pericon, Oax.....**S. periconia**
- 3. Petioles 0.5-1.0 cm long; Pue, Oax**S. ramosa**
- 4(2). Upper stems pubescent with branched hairs**S. melissodora**
- 4. Upper stems variously pubescent but w/o branched hairs (5)
- 5(3). Corolla tubes 3-5 mm long.....**S. ramosa**
- 5. Corolla tubes 5-13 mm long...(6)
- 6. Corolla tubes 7-13 mm long...(13)
- 6. Corolla tubes 5-7 mm long, or leaves 1.0-1.5 times as long as wide...(7)
- 7. Calyx densely glandular-pubescent.....**S. melissodora**
- 7. Calyx not glandular-pubescent...(8)
- 8. Larger leaves 12-15 cm long; Oax.....**S. occidua**
- 8. Larger leaves 1-5 cm long...(9)

- 9. Petioles 5-10 mm long; blades 3-5 cm long...(11)
- 9. Petioles 1-5 mm long; blades 1-2 cm long; Pue...(10)
- 10. Calyx glandular-pubescent.....**S. paupercula**
- 10. Calyx densely white-woolly.....**S. boegei**
- 11(9). Leaves 1-2 times as long as wide; Nue, Tam to Hid.....**S. keerlii**
- 11. Leaves 2-3 times as long as wide; Mex to Gue.....(12)
- 12. Calyx densely ivory-white pubescent.....**S. breviflora**
- 12. Calyx not as described in the above.....**S. melissodora**
- 13(6). Corolla tubes white, 10-13 mm long.....**S. semiatrata**
- 13. Corolla tubes purple, 7-9 mm long.....(14)
- 14. Foliage eglandular-pubescent.....(17)
- 14. Foliage glandular-pubescent.....(15)
- 15. Calyx 5-6 mm long; corolla tube 7-9 mm long.....**S. rupicola**
- 15. Calyx 6-7 mm long; corolla tube 6-7 mm long.....(16)
- 16. Plants of Nayarit.....**S. tepecensis**
- 16. Plants of Sinaloa.....**S. aequidistans**
- 17(14). Calyx minutely glandular; Pue.....**S. pannosa**
- 17. Calyx white-villous; Nue, Tam, Dur, San, Hid.....**S. keerlii**

SALVIA AEQUIDISTANS Fernald, Proc. Amer. Acad. Arts 35: 512. 1900. **Map 1**

S Sin, between Rosario and Colomas (type material) and San Ignacio, ca 400 m; Jul.

As indicated by its author, the species “Scarcely differs from *S. tepicensis* save in the more lax habit, longer petioles, less dense pubescence and thinner leaves.”

SALVIA BOEGEI Ramamoorthy, J. Arnold Arb. 65:138. 1984.

Map 2

Known only from Puente de Dios Molcaxac, Pue, 1800 m;
Sep.

According to its creator, the species "can be recognized immediately by its articulated petiole, almost capitate verticels [of flowers], and woolly white calyx." The relatively small leaves with short petioles also distinguish the species, the latter characters suggesting a close relationship with *S. paupercula*, which is readily distinguished from *S. boegei* by its glandular vestiture.

SALVIA BREVIFLORA Moc. & Sesse ex Benth., Lab. Gen. Sp. 274. 1833. **Map 2**

Salvia albicans Fernald

Salvia nelsonii Fernald

Mic, Mex, Mor, Pue and Gue, calcareous hillsides with juniper, 1000-1500 m; Aug-Oct.

This is an attractive blue-flowered shrub 1-3 m high having white-woolly compacted spikes

My synonymy follows that of Epling (1939).

SALVIA KEERLII Benth., Lab. Gen. Sp. 263. 1833. **Map 3**

Nue, Tam, San, Gua, Que?, Mic, Mex and Hid, pine-oak forests, 2400-3000 m, Aug-Oct.

Salvia keerlii in northeastern Mexico is a relatively common blue-flowered shrub 1-2 m high; I have not seen specimens from the state of Durango, as reported by Epling (1939).

SALVIA MELISSODORA Lag., Gen. et Sp. 2. 1816. **Map 4**

Salvia dugesii Fernald

Salvia scorodoniifolia Poir.

Salvia scordoniifolia var. *crenaea* Fernald

A widespread highly variable species distributed throughout most of central Mexico in relatively dry bushy habitats, 500-2500 m; Aug-Mar.

As well noted by Epling, this taxon has two pubescent forms: plants with glandular hairs, and those with branched hairs. The name *S. dugesii* has been applied to the latter.

SALVIA OCCIDUA Epling, Repert. Spec. Nov. Regni Veg. Beih. 110: 173. 1939. **Map 2**

Oax, coastal areas near San Miguel del Puerto, known to me only from Liebmans collections cited by Epling.

A poorly collected taxon, readily distinguished from closely related taxa by its large foliage.

SALVIA PANNOSA Fernald, Proc. Amer. Acad. Arts 40: 54. 1905. **Map 3**

Southern Pue and closely adjacent Oax, xeric shrub lands with Juniperus, 1200-3000 m; Jul-Oct.

Said to be a locally abundant purple-flowered subshrub 1-2 m high, relatively distinctive because of its bicolored leaves, the blades of which are rather lanceolate and truncate to subcordate at the base.

SALVIA PAUPERCULA Epling, Repert. Sp. Nov. Regni Veg., Beih. 110: 173. **Map 4**

This is a poorly known taxon, reportedly from near Fort de Guadalupe and Rancho Posada, Pue, the type (US) collected by F. Arsenault in 1909.

Except for its vestiture this taxon appears closely related to *S. boegeri*, so noted under the latter.

SALVIA PERICONA B.L. Turner, sp. nov. **Fig. 1 Map 5**

Salvia ramosa Brandegeesimiles sed differt foliis multo majoribus valde bicoloribus ac plerumque cordatis, petiolo longioribus (2-3 cm longis vs 0.5-1.0 cm), et corollis majoribus albis (vs. azureis).

Perennial herbs or subshrubs to at least 0.5 m high. **Stems** purplish, pubescent with peculiar erect scattered 2-3 celled trichomes 0.2-0.3 mm high, beneath these an understory of minute glandular hairs. **Leaves** (the larger) 5-8 cm long, 2-5 cm wide; petioles 2-3 cm long, having a distinct disarticulation scar at the base; blades deltoid to cordate, bicolored, the lower surface densely white-tomentose, the upper dark green and rugose, beset with small hairs throughout, the margins irregularly serrulate. **Spikes** terminal, 5-15 cm long, the flowers arranged 4-10 at interrupted nodes. **Floral bracts** (uppermost) broadly ovate, 6-8 mm long, 4-5 mm wide, soon deciduous. **Flowering calyces** 8-9 mm long, pubescent like the stems, the upper lip ca 3 mm long; pedicels 2-4 mm long. **Corollas** white, smooth within; tube 8-9 mm long; upper lip ca 4 mm long; lower lip 4-5 mm long, reflexed. Anthers not excurrent; filaments ca 3 mm long; anthers ca 1.5 mm long. Style pilose, the upper branch recurved, 2-3 times as long as the lower.

TYPE: MEXICO. OAXACA: **Mpio. Tamazulapan**, Cerro Pericon, 5 km al N de San Pedro Nopala, "Suelo obscuro derivido de roca ignea." 2000 m, 6 Jul 1986, *Abisai Garcia M. 2342* (with D. Frame, P. Tenorio and A. Salinas) (holotype: TEX).

ADDITIONAL SPECIMEN EXAMINED: Same locality as Type, "Bosque de Encino con elementos de Matorral xerofilo, 2350 m, 13 Nov 1985, *Ramamoorthy 4778* (TEX).

The label of *Ramamoorthy 4778* describes the plant as a white-flowered herb 0.5 m high; the species name is taken from the Sierra to which it is possibly confined. *Ramamoorthy* apparently also recognized the taxon as new when collected, to judge from his annotation on the sheet concerned.

SALVIA RAMOSA Brandegee, Zoe 5: 255. 1908. **Map 4**
Salvia lantanifolia Mart. & Gal. ?
Salvia variana Epling

Southern Pue and closely adjacent Pue in dry shrublands with *Juniperus*, 1800-3000 m; Sep-Nov.

As pointed out in detail by Epling (1939), *S. ramosa* is a highly variable taxon, especially in pubescence, possessing calyces

with only branched hairs, or else pubescent throughout with multiseptate glandular trichomes. He also noted that *S. ramosa* was "Scarcely to be distinguished from *S. mellissodora* save in the smaller leaves, smaller flowers and finer pubescence." I am unable to distinguish Epling's *S. variana*, the latter reportedly having somewhat larger corollas and longer petioles than typical *S. ramosa*. Finally, it should be noted that the earlier name *S. lantanifolia* Mart. & Gal. might be better tagged upon the present, since its distribution and general habit, as judged by phototypes at TEX appear very similar to *S. ramosa*. Indeed, Brandege assigned the name *S. lantanifolia* to the type of *S. variana*.

SALVIA RUPICOLA Fernald, Proc. Amer. Acad. Arts 45: 420. 1910.

Map 1

Hid, vicinity of Ixmiquilpan, rocky hillsides; ca 2500 m; Jul-Aug.

Epling (1939) thought this taxon perhaps but a variety of his concept of *S. scordoniifolia*, but subsequently retained the species (Epling 1944).

SALVIA SEMIATRATA Zucc. in Abhandl. Akad. Muench. 1. 298. 1832. **Map 5**

Oax, where relatively common in pine-oak forests, 200-2500 m; Jun-Oct.

A very distinctive blue-flowered shrub 1-2 m high having mostly cordate, markedly rugose, leaves and relatively large flowers.

Epling (1939) treated this species as the sole member of his sect. *Atratae*. I think it better positioned within the sect. *Scorodonia*.

SALVIA TENORIOI Ramamoorthy ex B.L. Turner, **sp. nov.** **Fig. 2**
Map 5

Salvia ramosa Brandegeae similes sed differt corollas flavis (vs azureis vel purpuratis), floribus in paniculis spicatis dispositis, et vestimento calycini trichomatum glandulosorum (vs trichomatum ramosorum).

Shrubs 2-3 m high. **Stems** mostly pubescent with white, recurved hairs ca 0.3 mm high. **Leaves** (newly produced among the upper nodes) ovate to deltoid, markedly rugose, their margins crenulate; petioles 1-2 mm long. **Flowers** arranged in paniculate interrupted spikes, the latter mostly 4-6 cm long, the terminal panicle ca 30 cm high, 25 cm wide. **Floral bracts** (uppermost) lanceolate, 2-4 mm long, glandular-pubescent, soon deciduous. **Flowering calyces** 6-7 mm long, glandular-pubescent with viscid hairs ca 0.5 mm high; upper lip ca 1.5 mm long, 5-nerved; lower lip ca 1 mm long. **Corollas** yellow; tube more or less straight, 7-8 mm long, papillose and/or rugose within; upper lip ca 3 mm long; lower lip flabellate, reflexed, 3-4 mm long. **Anthers** not excurrent, attached near the throat of the tube; filaments 3-4 mm long, markedly flattened and recurved or twisting at maturity. **Style** sparingly pilose, more so below, the upper branch 2-3 times as long as the lower. **Nutlets** ovoid, glabrous, ca 2.5 mm long, 1.5 mm wide.

TYPE: MEXICO. PUEBLA: Mpio. Teontepec, "14 km al NW de Teontepec, brecha a Nopala...Matorral calcicola mixta...Suelo negro sobre roca caliza." 16 Nov 1985, *P. Tenorio L. & G. Dieringer 10648* (holotype TEX).

According to label data the flowers are yellow, and the plants are said to be abundant shrubs 2-3 m high. The species is named for its collector, Pedro Tenorio, this suggested on the type itself by Ramamoorthy soon after its collection. According to Dr. Fernando Chiang, Pedro is a diligent collector and photographer of the Mexican flora who formerly worked at MEXU. He assembled over 20,000 numbers from throughout Mexico, and is well known for his collections from the area of Caltepec, Puebla where he was born and raised.

Yellow-flowered *Salvias* are quite rare in Mexico, as noted by Ramamoorthy (1984). In his description of the yellow-flowered *S. tuxtlensis* he stated, "Of the estimated 275 species in Mexico only three have yellow flowers." Actually, including *S. tuxtlensis* and the present novelty, five yellow-flowered species are known, Ramamoorthy having been unaware of the lovely *S. madrensis* of Sinaloa. Epling (1939) placed the yellow-flowered species known to him (*S. aspera* Kunth, *S. madrensis*, *S. subhastata*, and *S. hidalgensis* Mir.) in four Sections.

Ramamoorthy did not assign his novelty to a Section but allowed as to how it might belong to yet another monotypic Section. By implication, in my Latin diagnosis I have tentatively assigned *S. tenorioi* to the sect. *Scordonia*, the plant concerned having the general habit and vegetative features of that assemblage.

SALVIA TEPICENSIS Fernald, Proc. Amer. Acad. Arts 45: 420.

1910. **Map 1**

Salvia scordoniifolia var. *subsessilis* Benth.

Nay and Col, mixed mesophytic forests along the Pacific coast, 300-900 m, Jun-Aug.

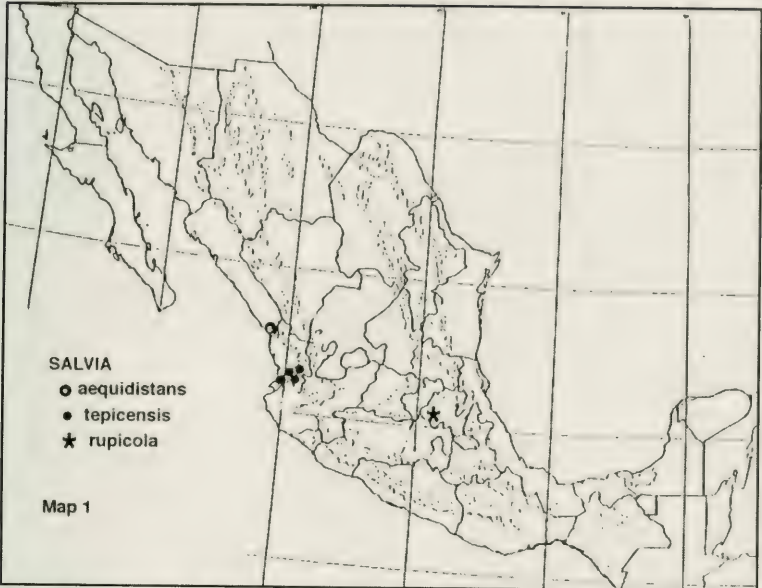
This species is doubtfully placed in the sect. *Scordonia*, although maintained by Epling (1939); at least it appears to lack the distinctive disarticulation scar at the base of petioles found in most of the other taxa of the complex. Regardless, Epling positioned *S. tepicensis* in sect. *Scorodonia*, along with *S. aequidistans*, the two scarcely distinguishable

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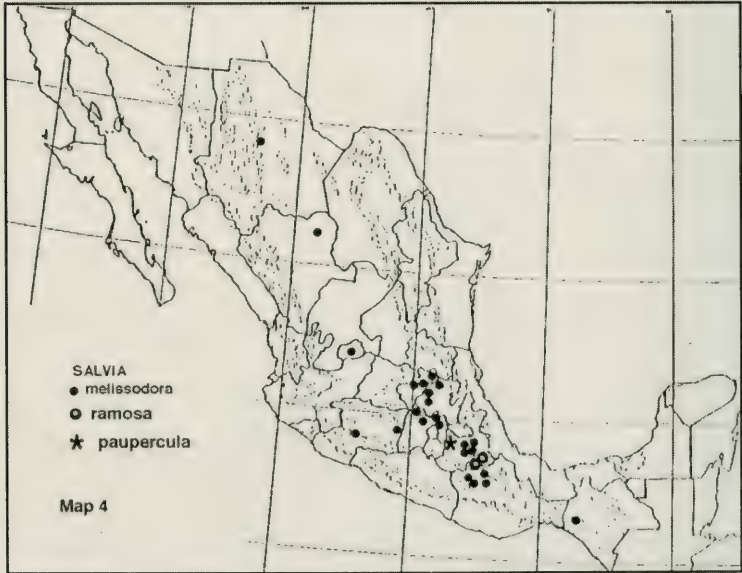






Fig. 1. Holotype of *Salvia pericon*.



Fig. 2. Holotype of *Salvia tenorioi*.

KEYS TO THE FLORA OF FLORIDA: 22,
DICERANDRA (LABIATAE)

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ABSTRACT

Dicerandra (Labiatae) is represented in Florida by three species: one, *D. densiflora*, is monotypic; one, *D. linearifolia*, consists of two varieties; and one, *D. frutescens*, is believed best interpreted to be formed of 7 varieties. *Dicerandra frutescens* var. **christmanii**, var. **cornutissima**, var. **immaculata**, var. **modesta**, var. **savannarum**, and var. **thinicola** are recognized as new combinations. *Dicerandra densiflora* and *D. frutescens* are endemic to Florida. *Dicerandra frutescens* (typical) and certain of its varieties are rated as endangered. An amplified key is given to the Florida taxa. *Phytologia* 91(2): 270-276 (August, 2009).

KEY WORDS: *Dicerandra*, Labiatae, Florida flora.

The task of the present number is to formalize the change in rank of certain taxa within the woody mint, *Dicerandra* (Labiatae), as needed for uniform presentation within an amplified key to the Florida species.

Soon after arriving in Florida in 1958, the present author discovered in the herbarium (FLAS) specimens that seemed not to belong where they had been filed. Many had been collected by James B. McFarlin (1901-1969), from near Sebring, Highlands Co., and others from elsewhere in the peninsula. Trips to collection sites quickly found additional populations, and a small folder was assembled of notes and information that might eventually become meaningful. But in 1961 a request came from Lloyd H. Shinnery (SMU), for the

loan of the genus *Dicerandra*. With the advice of Erdman West, a selection of materials was made and dispatched to Texas.

The result was Shinnery's publication (Sida 1: 89-91, 1962) of a synopsis of the genus, with the unnamed materials now named *Dicerandra frutescens*. Their distribution was from throughout the peninsula and showed no variances sufficient to bring attention.

But within the year Olga Lakela, then in Tampa (USF), found plants near the eastern shore of the peninsula which lacked dots on the lip of the corolla; she named it *Dicerandra immaculata* (Sida 1: 184-185, 1963). Lakela was followed by Robin Huck who ranged widely, throughout Florida, Georgia, and the Carolinas; she found further variation in north-central Florida which she named *D. cornutissima* (Phytologia 47: 313-316, 1981). A student of the peninsular scrub flora, Steven Christman, then encountered a different form in Highlands County, and a team of 6 investigators pooled their resources to name it *D. christmanii* (Huck et al., Syst. Bot. 14: 197-213, 1989). A bryologist, Harvey Miller, with close contacts among vascular systematists, was inspired to find and describe another species, *D. thimicola* (Phytologia 75: 185-189, 1993). A degree of order and understanding was brought to this burgeoning profusion of new entities by Huck & Chambers (Edinb. J. Bot. 54: 217-229, 1997) who found different ploidy levels and distinct though often overlapping ranges for all described taxa. Loose ends were tidied up by Huck (Novon 11: 417-420, 2001), in describing 2 infraspecific entities, *D. frutescens* ssp. *modesta*, and *D. immaculata* var. *savannarum*. A comprehensive summary of the entire nine-species genus, utilizing total genomic DNA and permitting the drawing of phylogenetic conclusions, was then assembled by L. O. Oliveira, R. B. Huck, M. A. Gitzendanner, W. S. Judd, D. E. Soltis and P. S. Soltis (Amer. J. Bot. 94: 1017-1027, 2007).

This body of recent literature is far too extensive for summation here. Clearly, past geologic changes have served to isolate small populations of the woody mint that Shinnery called *D. frutescens*, permitting alteration in gene frequency and small changes in morphology that astute observers have now been able to detect.

Perhaps in time there will come an understanding of sea-level and climate changes that have influenced not only these taxa but other members of the complex Florida flora.

But from the standpoint of a conventional taxonomist, either lacking knowledge of source or denying himself the unearned luxury of determining a species based almost entirely on where it was found, the described morphological differences are insufficient. Other species in related genera are not so finely dissected, and it is inappropriate that these taxa bear names well beyond the usual meaning of their morphological basis. In essence, Lloyd Shinnery was right; the woody mints of this group are a single species, *Dicerandra frutescens*.

Dicerandra frutescens L. H. Shinnery var. *christmanii* (R. B. Huck & W. S. Judd) D. B. Ward, comb. et stat. nov. Basionym: *Dicerandra christmanii* R. B. Huck & W. S. Judd, in R. B. Huck, W. S. Judd, W. H. Whitten, J. D. Skean, R. P. Wunderlin, & K. R. Delaney, Syst. Bot. 14: 198. 1989. TYPE: United States, Florida, Highlands Co., Sebring, 10 Sept 1987, *Hansen & DeLaney 4825* (holotype, FLAS; isotypes, A, BHO, DUKE, F, FLAS, FTG, GA, K, MO, MSC, NCU, NY, SMU, TEX, UC, US, USF).

Dicerandra frutescens L. H. Shinnery var. *cornutissima* (R. B. Huck) D. B. Ward, comb. et stat. nov. Basionym: *Dicerandra cornutissima* R. B. Huck, Phytologia 47: 313. 1981. TYPE: United States, Florida, Marion Co., Fla. 484 & I-75, 19 Sept 1980, *Huck 2436* (holotype, NCU).

Dicerandra frutescens L. H. Shinnery var. *immaculata* (O. Lakela) D. B. Ward, comb. et stat. nov. Basionym: *Dicerandra immaculata* O. Lakela, Sida 1: 184. 1963. TYPE: United States, Florida, Indian River Co., U.S. 1, 30 Sept 1962, *Lakela 25440* (holotype, USF).

Dicerandra frutescens L. H. Shinnery var. *modesta* (R. B. Huck) D. B. Ward, comb. et stat. nov. Basionym: *Dicerandra*

frutescens L. H. Shinnars ssp. *modesta* R. B. Huck, Novon 11: 417. 2001. TYPE: United States, Florida, Polk Co., Dundee, 9 Sept 1999, *Huck 5555* (holotype, FLAS; isotypes, MO, USF).

Dicerandra frutescens L. H. Shinnars var. *savannarum* (R. B. Huck) D. B. Ward, comb. nov. Basionym: *Dicerandra immaculata* O. Lakela var. *savannarum* R. B. Huck, Novon 11: 419. 2001. TYPE: United States, Florida, St. Lucie Co., Savannas State Preserve, 26 Oct 1996, *Huck 5492* (holotype, FLAS; isotype, MO).

Dicerandra frutescens L. Shinnars var. *thinicola* (H. A. Miller) D. B. Ward, comb. et stat. nov. Basionym: *Dicerandra thinicola* H. A. Miller, Phytologia 75: 185. 1993. TYPE: United States, Florida, Brevard Co., Brandt Road, 5 Nov 1987, *Reifler s.n.* (holotype, MU; isotypes, USF, FTU).

The present paper, intended to address a subset of plant names which bear names at a level above that justified by their morphological differences, was initially limited to concern of the approach to take with *Dicerandra*, where a cottage industry has arisen to identify and name further variants of this heretofore unimportant woody mint. Every one of the many authors is (or has been -- Olga Lakela, 1890-1980; Lloyd Shinnars, 1918-1971) a close associate or friend of the present author. One trusts that friendship will not be weakened by the present author's nomenclatural diminution of their discoveries.

DICERANDRA Benth. Scrub Balmsⁱ

1. Annual (or persisting a second year and developing a thickened collar at soil line, but not woody), unbranched or with few spreading to ascending branches well above base.
2. Peduncles absent or very short, the flowers numerous and crowded in axils of leaves or leafy bracts; anther horns abrupt, obtuse or barely acute. Pungently aromatic annual herb, to 0.4 m. Dry woods, pinelands, roadsides. North peninsula (Lafayette,

Suwannee, s. to Levy, Volusia counties); infrequent. Fall. Endemic. **Dicerandra densiflora** Benth in DC.

2. Peduncles (except at upper nodes) evident, usually well developed, few-flowered, the inflorescences rather loose; anther horns subulate, acuminate. Annual herb, to 0.4 m. Dry pineland, dry open hammocks. Fall. **Dicerandra linearifolia** (Ell.) Benth.

- a. Corolla pale pink to white; anthers yellow; leaves narrow. West and central panhandle (e. to Leon, Wakulla counties); infrequent. **var. linearifolia**

- a. Corolla dark purple; anthers reddish-brown; leaves wider. Central and east panhandle (Jackson to Suwannee), disjunct eastward (to Alachua, Duval counties); infrequent.

var. robustior Huck

1. Perennial, woody below, with numerous erect to ascending branches from near base. Low shrub, to 0.6 m. Sand pine scrub. Summer-fall. Endemic. ENDANGERED (Federal, State listings).

SCRUB BALM.

Dicerandra frutescens Shinnery

- a. Corolla without spots; corolla tube smoothly curved.

- b. Habit upright; leaves narrowly oblanceolate, 1.5-4.0 mm. wide; corolla light lavender. East coast of peninsula (Indian River, St. Lucie counties); very rare. Endemic. ENDANGERED (Federal, State listings). [*Dicerandra immaculata* Lakela] Occasional white-flowered plants have been named forma **nivea** Lakela.

var. immaculata (Lakela) D. B. Ward

- b. Habit lax; leaves rhombic, 1.2-12.0 mm. wide; corolla vivid pink. East coast of peninsula (St. Lucie County); very rare (two populations, with 200 individual plants). Endemic. [*Dicerandra immaculata* var. *savannarum* Huck]

var. savannarum (Huck) D. B. Ward

- a. Corolla with spots or blotches, at least on upper petals; corolla tube abruptly bent.
- c. Corolla reddish-purple; anther spurs >1 mm. long.
- d. Leaves very narrow (± 1.0 mm. wide); style glabrous or with few hairs. North-central peninsula (Marion, Sumter counties); rare. Endemic. ENDANGERED (Federal, State listings). [*Dicerandra cornutissima* Huck]
var. **cornutissima** (Huck) D. B. Ward
- d. Leaves broader (± 1.3 mm. wide); style hispid. Eastern coastal peninsula (Brevard County: Titusville); very rare (three known populations). Endemic. ENDANGERED (State listing). [*Dicerandra thinicola* H. A. Miller]
var. **thinicola** (H. A. Miller) D. B. Ward
- c. Corolla white or cream; anther spurs <1 mm. long; style with numerous stiff hairs; leaves narrowly oblong (1.3-2.5 mm. wide).
- e. Anthers bright yellow, with few or no glands on connective; crushed leaves with wintergreen odor. Central peninsula (Highlands County: Sebring); very rare (five known populations). Endemic. ENDANGERED (Federal, State listings). [*Dicerandra christmanii* Huck & Judd]
var. **christmanii** (Huck & Judd) D. B. Ward
- e. Anthers white or lavender, with abundant glands on connective; crushed leaves with minty fragrance.

- f. Inflorescence with 1-2 flowers per cyme; corolla white or rarely pink. Central peninsula (Polk, Highlands County: Lake Placid); very rare (nine extant populations). Endemic. ENDANGERED (Federal, State listings). var. **frutescens**
- f. Inflorescence with 2-3 flowers per cyme; corolla pinkish white, turning pink with age. Central peninsula (Polk County: Davenport); very rare (two known populations). Endemic. [*Dicerandra frutescens* ssp. *modesta* Huck] var. **modesta** (Huck) D. B. Ward

i. This paper is a continuation of a series begun in 1977. The "amplified key" format employed here is designed to present in compact form the basic morphological framework of a conventional dichotomous key, as well as data on habitat, range, and frequency. Amplified keys are being prepared for all genera of the Florida vascular flora; the present series is restricted to genera where a new combination is required or a special situation merits extended discussion.

**INFRASPECIFIC VARIATION IN *HESPEROCYPARIS*
GOVENIANA AND *H. PYGMAEA*: ISSRS AND TERPENOID
DATA**

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ABSTRACT

Hesperocyparis (*Cupressus*) *goveniana* and putative *Cupressus goveniana* subsp. *gibsonensis* plus *H. pygmaea* were analyzed by Inter-Simple Sequence Repeats (ISSRs). The ISSRs analyses support the continued recognition *H. pygmaea* and *H. goveniana*, but the recognition of *Cupressus goveniana* subsp. *gibsonensis* Silba was not supported. *Phytologia* 91(2):277-286 (August, 2009).

KEY WORDS: *Hesperocyparis* (= *Cupressus*) *goveniana*, *Cupressus goveniana* subsp. *gibsonensis*, *H. pygmaea*, ISSR, Inter-Simple Sequence Repeats, terpenes, DNA fingerprinting, systematics.

The coastal California species of *Hesperocyparis* (= *Cupressus*) are often found in very small populations and several are endangered. The taxonomy of the western hemisphere cypresses has recently changed as DNA sequencing of the classical *Cupressus* species (Little et al., 2004; Little, 2006) demonstrated that the western hemisphere cypresses are a separate genus from the eastern hemisphere cypresses. The eastern hemisphere cypresses maintained the name *Cupressus* and *Callitropsis* was applied to the western hemisphere cypresses plus *Callitropsis nootkatensis* (D. Don) Oersted ex D. P. Little. However, Debreczy, et al. (2009) argued, on morphological

grounds, that *Callitropsis nootkatensis* is a monotypic genus. Sequencing of two additional nuclear genes and petN-psbM (Adams et al., 2009) supported the recognition of *Callitropsis nootkatensis* as a monotypic genus. Thus, *Callitropsis* could not be applied to the western hemisphere cypresses, so Adams et al. (2009) erected a new genus, *Hesperocyparis*, for these cypresses.

Silba (2003) proposed the recognition of several new subspecies of *Cupressus abramsiana* Wolf: *C. a.* subsp. *locatellii* Silba, Eagle Rock, CA; *C. a.* subsp. *opleri* Silba, Bracken Brae Forest, Santa Cruz, CA; *C. a.* subsp. *neolomondensis* Silba, Majors Creek, CA; and *C. a.* subsp. *butanoensis* Silba, Butano Ridge, CA. *Cupressus a.* subsp. *butanoensis* was recognized (Adams et al., 2009) as *Hesperocyparis abramsiana* var. *butanoensis* (Silba) Bartel & R. P. Adams. Silba (2003) also proposed *C. goveniana* Gordon subsp. *gibsonensis* Silba and *C. macrocarpa* Hartw. subsp. *lobosensis* Silba. However, the proposed new subspecies are morphologically rather indistinct. If these new subspecies are accepted, then these taxa need to be considered under the endangered species act.

Adams and Bartel (2009) examined the volatile leaf oils of *H. goveniana* (Gordon) Bartel, *C. g.* subsp. *gibsonensis* Silba and *H. pygmaea* (Lemmon) Bartel (Fig. 1). The leaf oils of these taxa appear to separate them into 2 groups (Fig. 1) composed of *H. pygmaea* and *goveniana* - *gibsonensis*. There was some variation among the three populations of *H. pygmaea* (Fig. 1) and a hint of differences between *goveniana* and *gibsonensis*. These differences appear to be in the nature of geographic variation. Adams and Bartel (2009) concluded that, based on the leaf terpenoids, there was insufficient evidence to support the recognition of Silba's *Cupressus goveniana* subsp. *gibsonensis*.

To gather additional genetic information about the validity of these subspecies, analyses using Inter-Simple Sequence Repeats (ISSRs) were conducted. It should be noted that the leaf samples utilized in the present study were taken from the same trees analyzed by Adams and Bartel (2009).

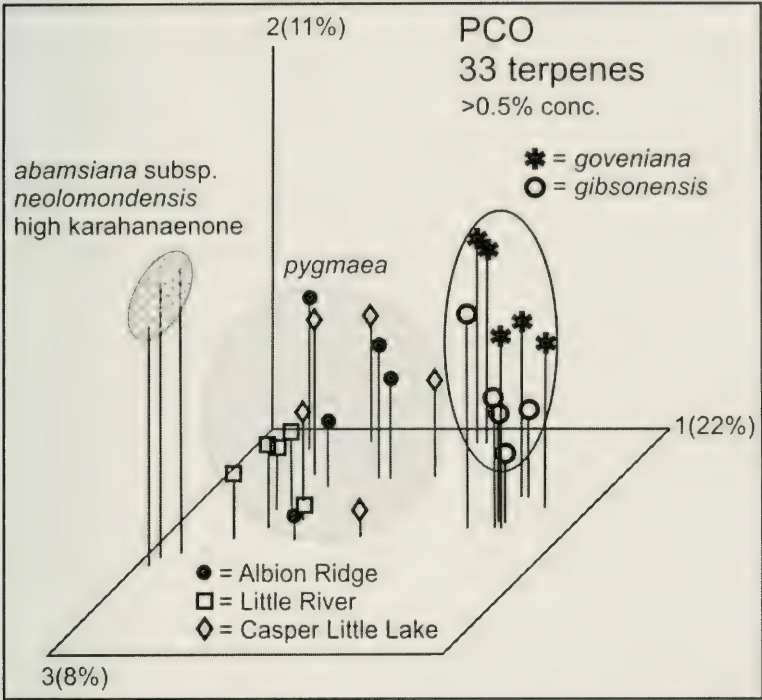


Figure 1. PCO of *Hesperocyparis goveniana*, *Cupressus* g. subsp. *gibsonensis*, *H. pygmaea* plus three plants of *C. abramsiana* subsp. *neolomondensis* (from Adams and Bartel, 2009).

MATERIALS AND METHODS

Plant material - Specimens used in this study: *H. abramsiana* (C. B. Wolf) Bartel, Bonny Doon, Santa Cruz Co., CA, Bartel 1598a-e; *H. abramsiana* var. *butanoensis*, Pescadero Creek County Park, Butano Ridge Fire Rd., San Mateo Co., CA, Bartel 1605a-e.; *C. abramsiana* subsp. *locatellii* Silba, Eagle Rock, Santa Cruz Co., CA, Bartel 1599a-e; *C. abramsiana* subsp. *neolomondensis* Silba, Wilder Ranch State Park, Santa Cruz Co., CA, Bartel 1604a-e; *C. a.* subsp. *opleri* Silba, Bracken Brae, Santa Cruz Co., CA, Bartel 1600a-e; *H. goveniana*, SFB

Botanical Reserve, Monterey Co., CA, *Bartel 1596a-e*; *C. goveniana* subsp. *gibsonensis* Silba, Point Lobos Ranch, Monterey Co., CA, *Bartel 1595a-e*; *H. pygmaea*, Albion Ridge, Mendocino Co., CA, *Bartel 1601a-e*; Little River Airport, *Bartel 1602a-e*; Casper Little Lake Rd., CA, *Bartel 1603a-e*; *C. macrocarpa* subsp. *lobosensis* Silba, Point Lobos State Reserve, Allan Memorial Grove, Monterey Co., CA, *Bartel 1593a-e*, East Grove, *Bartel 1594a-e*; *H. macrocarpa* (Hartw.) Bartel, Crocker Grove, 100 m n of 17 Mile Drive and Madre Lane intersection, Monterey Co., CA, *Bartel 1597a-e*. Bartel specimens are held in his personal herbarium in Carlsbad, CA.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia, CA). ISSR primers were purchased from the University of British Columbia (5'-3 seq., annealing temperature used'): 807: AGA GAG AGA GAG AGA GT (50°C), 808: AGA GAG AGA GAG AGA GC (50°C), 811: GAG AGA GAG AGA GAG AC (50°C), 812: GAG AGA GAG AGA GAG AA (50°C), 836: AGA GAG AGA GAG AGA GYA (54°C), 840: GAG AGA GAG AGA GAG AYT (54°C), 841: GAG AGA GAG AGA GAG AYC (54°C), 847: CAC ACA CAC ACA CAC ARC (58°C), 861: AGC AGC AGC AGC AGC (58°C), 881: GG TGG GGT GGG GTG (50°C), 886: VDV CTC TCT CTC TCT CT (50°C), 887: DVD TCT CTC TCT CTC TC (54°C), 895: AGA GTT GGT AGC TCT TGA TC (50°C), 900: ACT TCC CCA CAG GTT AAC ACA (50°C).

PCR stock solutions (Taq, primer, and buffer) were made in bulk so that all the PCR reaction tubes for a primer were prepared using the same bulk stock. This is a critical factor for minimizing variation in band intensities from sample to sample (see Adams, Flournoy and Pandey, 1998, for protocols to minimize PCR band variation). PCR was performed in a volume of 15 µl containing: 7.5 µl Epi-Centre 2X buffer E (containing 0.4 mM of each dNTP, final conc. = 0.2 mM), 0.75 µl primer (0.6 µM final conc.), 0.75 µl Epi-Centre Fail-Safe Taq (0.75 unit/rxn.), 6 µl genomic DNA (0.3 ng/rxn.). A negative control PCR tube containing all components, but no genomic DNA, was run with each primer to check for contamination. DNA amplification was performed in an MJ Programmable Thermal Cycler (MJ Research,

Inc.). Samples were run in duplicate to insure reproducibility (Adams, Flournoy and Pandey, 1998). A temperature profile was obtained for each well of the thermocycler to be sure that no variation existed among wells in the heating/ cooling block. The thermal cycle used was: 94° C (1.5 min) for initial strand separation, then 39 cycles of 91° C (1 min), 50° C (or 54° C or 58° C, see above) (2 min), 72° C (2 min). Two additional steps were used: 91° C (1 min), 50° C (or 54° C or 58° C) (2 min) and 72° C (5 min) for final extension. The temperature inside a PCR tube containing 15 µl buffer was monitored with a temperature probe, quantitated and printed for each step for each of the 40 cycles for every PCR run (Adams, Flournoy and Pandey, 1998) to insure that each cycle met temperature specifications and that each PCR run was exactly the same. Amplification products were analyzed by electrophoresis on 1.5% agarose gels, 70V, 55 min, and detected by staining with ethidium bromide. The gels were visualized over UV light and scanned to digital images. The digital images were size normalized in reference to pGem® DNA size markers before band scoring. Bands were scored as present (4 = faint, 5 = bright, 6 = v. bright) and absent (0). Bands that were inconsistent in replicate analyses were not scored.

Associational measures were computed using absolute character state differences (Manhattan metric), divided by the maximum observed value for that character over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis (PCO) was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). It should be noted that problems of homology of RAPD DNA bands on agarose gels can be significant (Rieseberg, 1996), but these errors can be accounted for using multivariate statistical methods (PCO) (see Adams and Rieseberg, 1998). A minimum spanning diagram was constructed by selecting the nearest neighbor for each taxon from the pair-wise similarity matrix, then connecting those nearest neighbors as nodes in a network (Adams, et al. 2003).

RESULTS AND DISCUSSION

The use of 14 ISSR primers resulted in 98 scoreable bands among the taxa. PCO of the association matrix removed five significant eigenroots accounting for: 15.2, 14.0, 9.1, 7.1, and 6.2% of

the variance among the OTUs. It is noteworthy that there was considerable variation among individuals and this is reflected in the relatively small amount of variance that was extracted by the first 3 eigenroots.

Ordination of the taxa shows that *H. goveniana* and *H. pygmaea* are very well resolved from *H. abramsiana* and *H. macrocarpa* (Fig. 2).

There appears to be considerable variation among the *H. pygmaea* individuals (Fig. 2). In addition, there is also variation among the *goveniana* - *gibsonensis* individuals (Fig. 2).

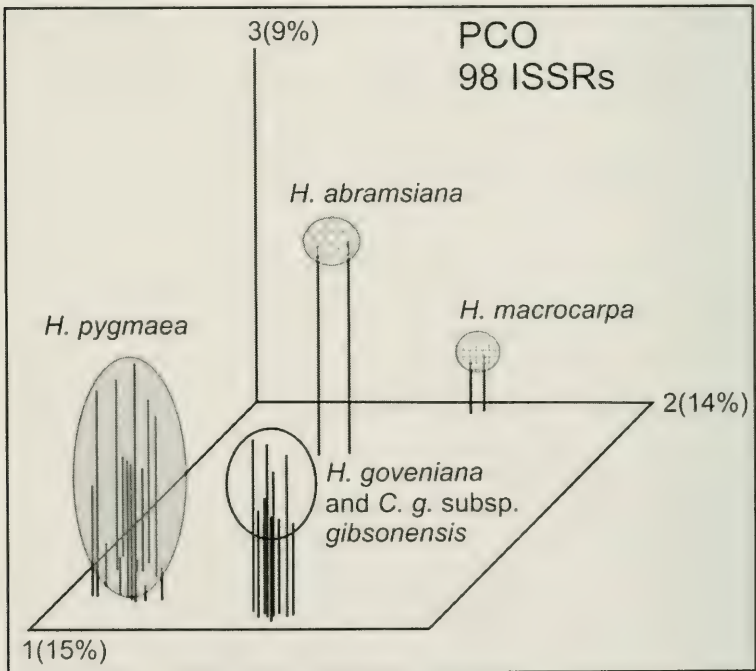


Figure 2. PCO of *H. goveniana*, *Cupressus goveniana* subsp. *gibsonensis*, and *H. pygmaea* with exemplars of *H. abramsiana* and *H. macrocarpa*.

In order to better visualize the variation among populations of *H. pygmaea*, a new PCO was performed which contained only the *H. pygmaea* individuals plus two exemplar *H. goveniana* samples. The PCO using 78 ISSRs resulted in four eigenroots accounting for 16.6, 12.8, 10.0, and 8.9% of the variance among individuals. Clearly there are considerable differences among *H. pygmaea* individuals (Fig. 3). There is a trend for the Casper Little Lake population (plus 2 individuals from the Albion Ridge population) to cluster together (Fig. 3). The balance of the Albion Ridge and Little River population plants are interspersed (Fig. 3). There was also a trend in the terpenoids to subdivide these populations (Fig. 1), but the pattern is slightly different

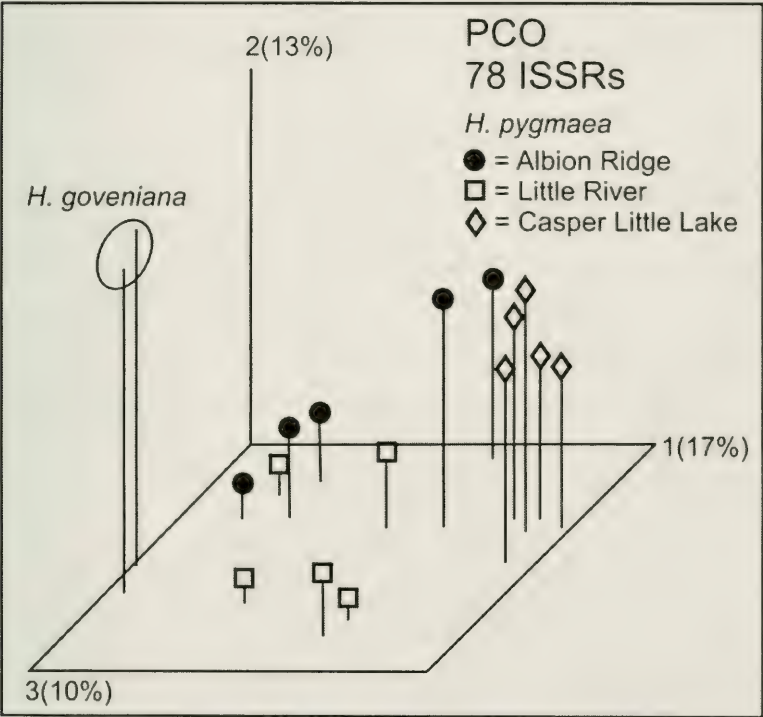


Figure 3. PCO based on 78 ISSRs for *H. pygmaea*. Two individuals of *H. goveniana* were included in the PCO.

than in the ISSR data (Fig. 3). It seems likely that these populational differences are normal geographic variation and should not to be recognized as separate taxa.

The variation between *H. goveniana* and *C. g.* subsp. *gibsonensis* was further examined using 75 ISSRs for their ten samples plus two individuals of *H. pygmaea*. PCO resulted in four eigenroots that accounted for 23.1, 19.5, 11.7, and 10.2% of the variance among these OTUs. Ordination reveals (Fig. 4) that *H. goveniana* and putative *C. g.* subsp. *gibsonensis* form a large group, with some separation between the taxa. However, as these putative subspecies are each in

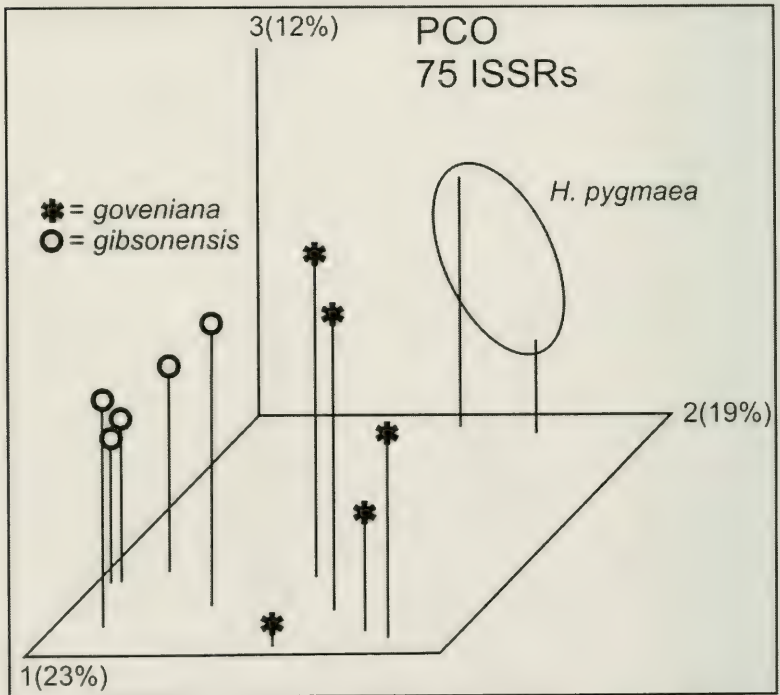


Figure 4. PCO of *H. goveniana* and *C. g.* subsp. *gibsonensis* individuals, plus two exemplars of *H. pygmaea*.

distinct (and disjunct) populations, geographic differentiation could well explain this clustering.

In summary, taking both the terpenoid and ISSR data into consideration, there appears to be sufficient genetic differentiation to support the recognition of *H. pygmaea*, but there is insufficient differentiation to support the recognition of Silba's *Cupressus goveniana* subsp. *gibsonensis*.

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INFRASPECIFIC VARIATION IN *HESPEROCYPARIS*
ABRAMSIANA: ISSRS AND TERPENOID DATA

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ABSTRACT

Five *Hesperocyparis* (*Cupressus*) *abramsiana* groves were analyzed by Inter-Simple Sequence Repeats (ISSRs). ISSRs analyses revealed geographical differentiation among the groves (= Silba's subspecies) with the Butano Ridge grove being the most distinct in both ISSRs and terpenoids. Combined ISSR and terpenoid data support the recognition of *Hesperocyparis. abramsiana* var. *abramsiana* and *Hesperocyparis abramsiana* var. *butanoensis* Bartel & R. P. Adams, comb. nov. The recognition of Silba's subsp. *locatellii*, *neolomondensis*, and *opleri* were not support by these data. *Phytologia* 91(2): 287-299 (August, 2009).

KEY WORDS: *Hesperocyparis* (= *Cupressus*) *abramsiana* var. *butanoensis*, *C. subsp. locatellii*, *C. subsp. neolomondensis*, *C. subsp. opleri*, ISSR, Inter-Simple Sequence Repeats, terpenes, DNA fingerprinting, systematics.

Hesperocyparis (= *Cupressus*, see Adams et al., 2009) *abramsiana* (C. B. Wolf) Bartel, widely known as Santa Cruz cypress, was listed (as *Cupressus abramsiana*) as an endangered species under the Endangered Species Act of 1973 (ESA) by the U.S. Fish and Wildlife Service (USFWS) in 1987. According to the ESA recovery plan developed for the species (USFWS 1998), *H. abramsiana* is restricted to five groves or populations (Fig. 1) that include a total of

5,100+ individuals that collectively occupy about 142 ha (356 acres) within a 24-km (15-mile) range in the Santa Cruz Mountains in Santa Cruz and San Mateo counties, California, USA. Using an ESRI shape file of the grove boundaries provided by the USFWS, McGraw (2007) clarified that the areal extent of the cypress groves depicted in the recovery plan actually include 41.28 ha (102.0 acres). McGraw (2007) estimated that the areal extent of the five groves totals only 25.87 ha (63.9 acres).

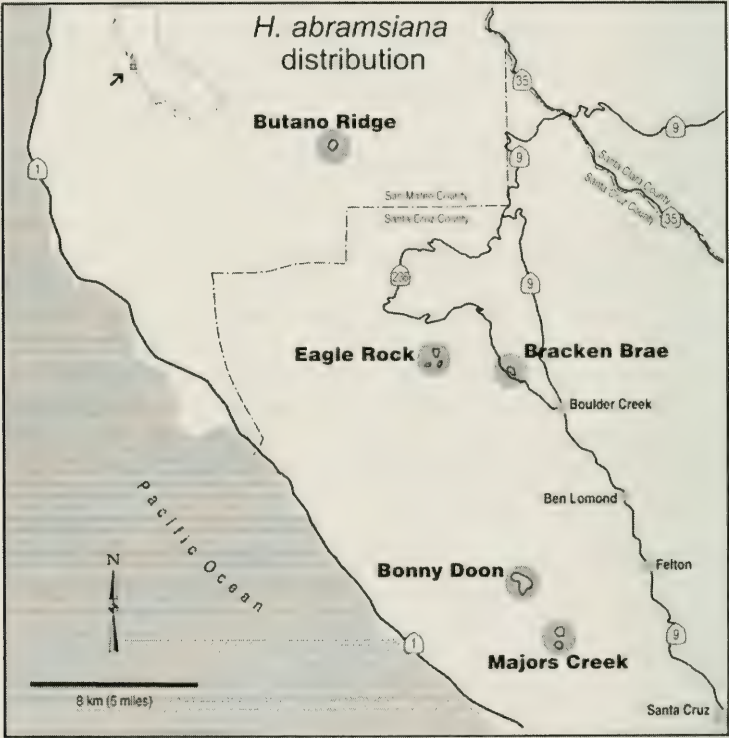


Figure 1. Distribution of *H. abramsiana* groves endemic to Santa Cruz and San Mateo counties, California, USA. Geographic limits of individual cypress stands within each grove, which are depicted in hatched polygons atop half-tone circles, were derived from Fig. 2 from McGraw (2007).

Using the ESA recovery plan and after visiting four of the five groves or populations, Silba (2003) subdivided *Cupressus abramsiana* into five subspecies with his descriptions of four new subspecies; *C. a.* subsp. *locatellii* Silba (restricted to the Eagle Rock grove in Santa Cruz County), *C. a.* subsp. *opleri* Silba (restricted to the Bracken Brae grove in Santa Cruz County), *C. a.* subsp. *neolomondensis* Silba (restricted to the Majors Creek grove in Santa Cruz County), and *C. a.* subsp. *butanoensis* Silba (restricted to the Butano Ridge grove in San Mateo County). According to Silba (2003), the nominate subspecies is restricted to the type locality of *H. abramsiana* (i.e., the Bonny Doon Grove in Santa Cruz County), which Wolf (1948) detailed as “on the southwest slope of Ben Lomond, a mountain 7/10 mi. east of the Bonnie Doon School, elevation 1600 feet.” Given the lack of a taxonomic key or a set of clear morphological characters to separate the subspecies, and the overall poor quality of the self-published article (e.g., orthographic errors, illegibility), Silba’s (2003) new subspecies seem to be morphologically indistinct and separated largely by collection locality.

Adams and Bartel (2009) examined the volatile leaf oils of *H. abramsiana* and Silba’s subsp. *butanoensis*, *locatellii*, *neolomondensis*, and *opleri* (Fig. 2). The leaf oils of these taxa appear to separate *H. abramsiana* into 2 groups (Fig. 2) composed of *butanoensis* and the remaining four groves. However, note that 3 trees of *butanoensis* had oils similar to *H. pygmaea* (Fig. 2, see also Table 1, Adams and Bartel, 2009). Adams and Bartel (2009) concluded that additional research was needed into the nature of infraspecific variation in *H. abramsiana*.

To gather additional genetic information about the validity of these subspecies, analyses using Inter-Simple Sequence Repeats (ISSRs) were conducted. The leaf samples utilized in the present study were taken from the same trees analyzed by Adams and Bartel (2009).

MATERIALS AND METHODS

Plant material - Specimens used in this study: *H. abramsiana*, Bonny Doon Grove, Santa Cruz Co., CA, Bartel 1598a-e; Butano Ridge Grove, (*butanoensis*), Pescadero Creek County Park,

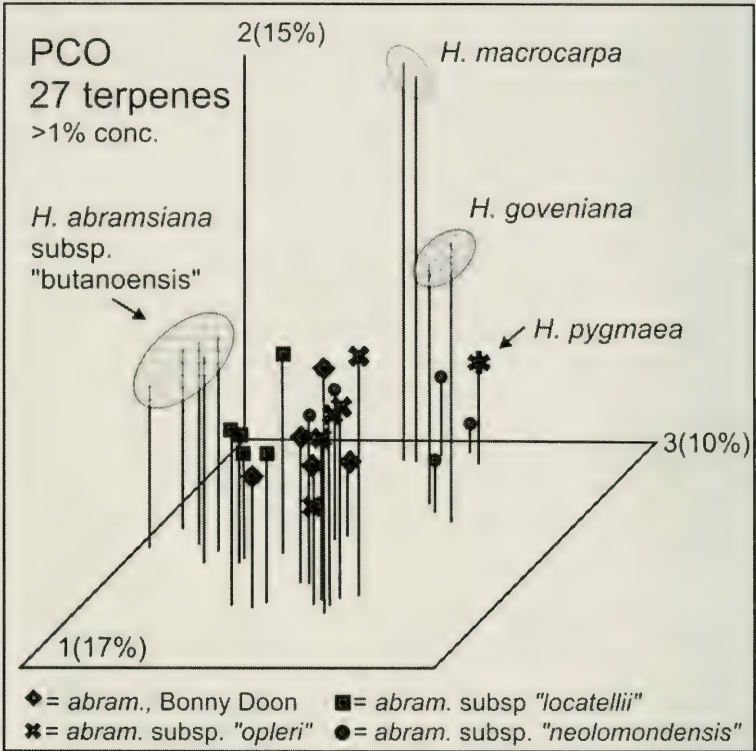


Figure 2. PCO of *H. abramsiana* and Silba's subspecies based on 27 terpenes. From Adams and Bartel (2009).

San Mateo Co., CA, *Bartel 1605a-e*; Eagle Rock Grove, (*locatellii*), Santa Cruz Co., CA, *Bartel 1599a-e*; Majors Creek Grove, (*neolomondensis*), Wilder Ranch State Park, Santa Cruz Co., CA, *Bartel 1604a-e*; Bracken Brae Grove, (*opleri*), Santa Cruz Co., CA, *Bartel 1600a-e*; *H. goveniana*, SFB Botanical Reserve, Monterey Co., CA, *Bartel 1596a-e*; *H. pygmaea*, Casper Little Lake Rd., CA, *Bartel 1603a-e*. Bartel specimens are held in his personal herbarium in Carlsbad, CA.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia, CA). ISSR primers were purchased from the University of British Colombia (5'-3 seq., annealing temperature used): 807: AGA GAG AGA GAG AGA GT (50°C), 808: AGA GAG AGA GAG AGA GC (50°C), 811: GAG AGA GAG AGA GAG AC (50°C), 812: GAG AGA GAG AGA GAG AA (50°C), 836: AGA GAG AGA GAG AGA GYA (54°C), 840: GAG AGA GAG AGA GAG AYT (54°C), 841: GAG AGA GAG AGA GAG AYC (54°C), 847: CAC ACA CAC ACA CAC ARC (58°C), 881: GG TGG GGT GGG GTG (50°C), 886: VDV CTC TCT CTC TCT CT (50°C), 887: DVD TCT CTC TCT CTC TC (54°C), 895: AGA GTT GGT AGC TCT TGA TC (50°C).

PCR conditions and numerical methods - see Adams and Bartel, 2009.

RESULTS AND DISCUSSION

The 12 ISSR primers resulted in 89 scoreable bands among the taxa. A minimum spanning network was constructed using the matrix of associations and is shown in figure 3. Individuals of each of the five groves of *H. abramsiana* cluster together. *Hesperocyparis goveniana* and *H. pygmaea* are well resolved (Fig. 3). However, the Butano Ridge grove actually clustered after *H. pygmaea* clusters (Fig. 3).

PCO of the association matrix removed six significant eigenroots accounting for: 13.74, 11.84, 10.51, 7.89, 7.70 and 6.67% of the variance among individuals. Ordination shows a similar pattern (Fig. 4) with *H. goveniana* and *H. pygmaea* being distinct from *H. abramsiana*. Most of the *H. abramsiana* groves are not resolved.

Removing *H. goveniana* and *H. pygmaea* from the data set and running a PCO focuses on the *H. abramsiana* groves. Ordination shows that all five *H. abramsiana* groves are resolved (Fig. 5). A minimum spanning network (dashed lines, Fig. 5) shows that Butano Ridge population is the most divergent grove (0.730). While three groves consist of a single largely distinct stand of *H. abramsiana*, the

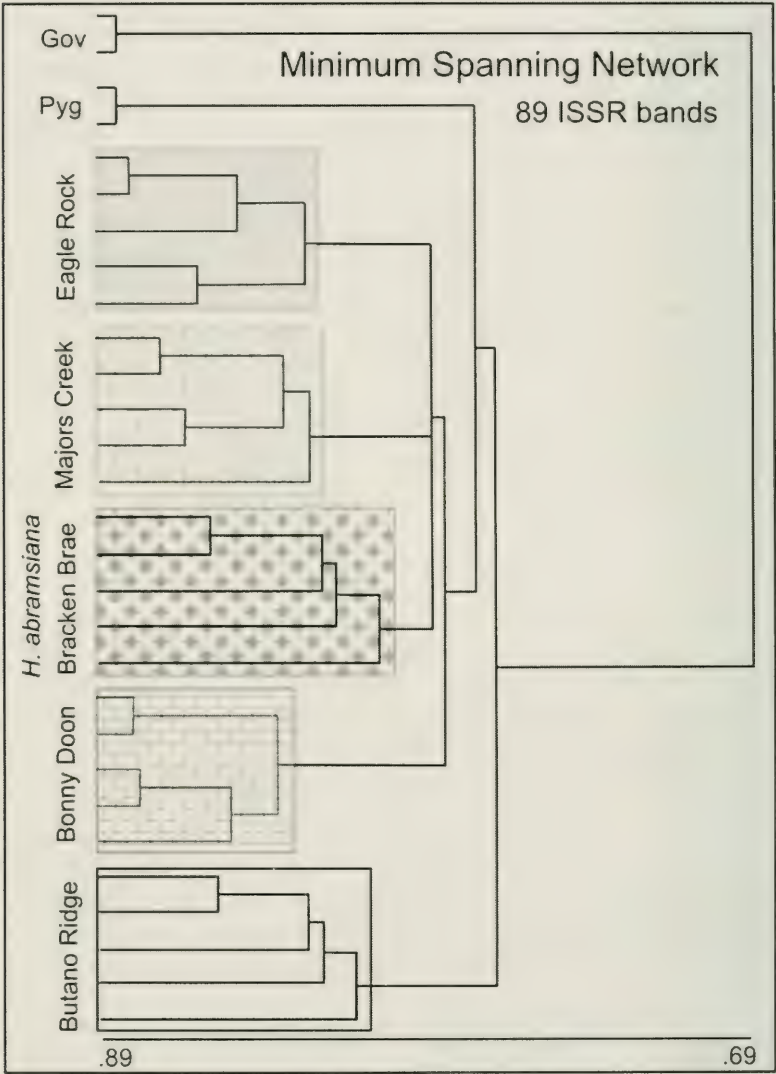


Figure 3. Minimum spanning network based on 89 ISSR bands. Gov = *H. goveniana*, Pyg = *H. pygmaea*.

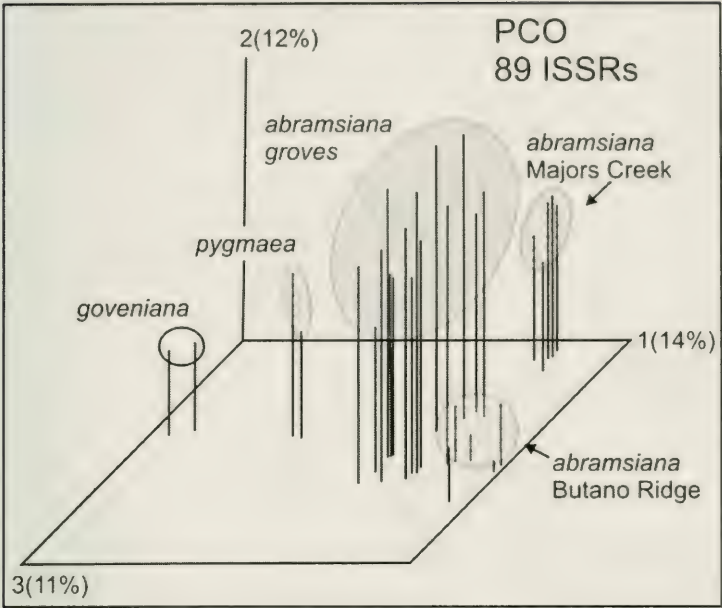


Figure 4. PCO based on 89 ISSR bands.

Eagle Rock and Majors Creek groves are made up of three and two stands respectively (USFWS 1998, McGraw 2007). Though Silba (2003) used the groves or populations as identified in the ESA recovery plan to delimit his five subspecies, he also included within *C. a.* subsp. *neolomondensis* two collections (*B-256* and *B-257*) from “a small grove of less than a dozen trees” within the Bonny Doon Ecological Reserve. This collection site apparently is between the Bonny Doon and Majors Creek groves, yet west of Laguna Creek and across Martin Road from the largest cypress stand and type locality. Much of the ecological reserve, including most of the cypress grove, burned in June 2008 in the 210-ha (520-acre) Martin Fire.

The five groves range from about 2.2 to 25.7 km (1.4 to 16.1 miles) apart, though the populations generally fall within three watershed basins. Going south to north, the Bonny Doon and

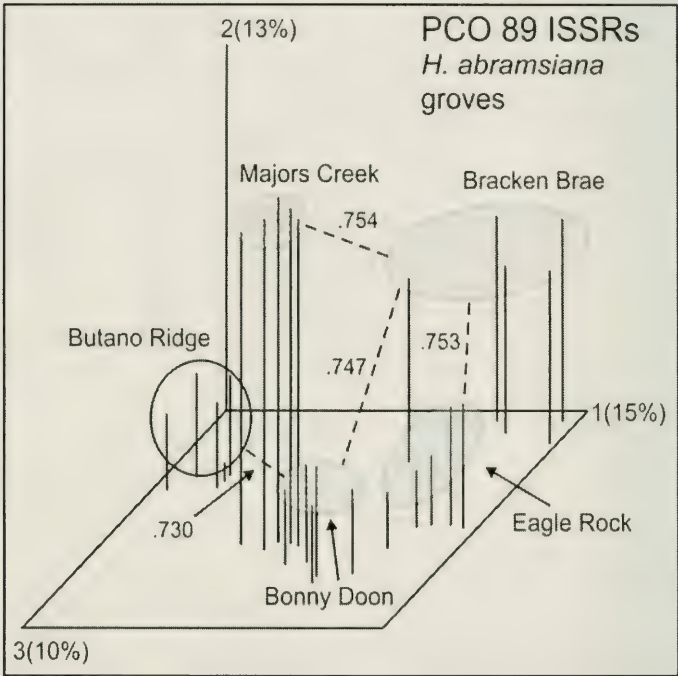


Figure 5. PCO based on 89 ISSR bands analyzing five *H. abramsiana* groves.

Majors Creek groves lie between Mill Creek (a tributary to San Vicente Creek) and Majors Creek with Laguna Creek bisecting both groves. The Eagle Rock and Bracken Brae groves largely fall within the watershed of Boulder Creek and its tributary Jamison Creek. The four southernmost groves drain generally south toward Santa Cruz or immediately to the northwest of Monterey Bay. However, the Butano Ridge grove, well isolated from the other groves, is located within the Butano Creek watershed, which drains west and empties into the estuary in Pescadero Creek State Beach. With this hydrology in mind, the four southernmost groves are very similar (0.747 - 0.754), while the divergence of the Butano Ridge grove (0.730) correlates with its relative isolation from the other populations.

In summary, both the terpenoids (Fig. 2) and ISSRs (Figs. 3-6) data show that the Butano Ridge grove is differentiated from the four southernmost *H. abramsiana* groves. Little variation was found among the four groves in the terpenoids (Fig. 2). The three individuals (Fig. 2) that had leaf oils similar to *H. pygmaea* were not similar to *H. pygmaea*

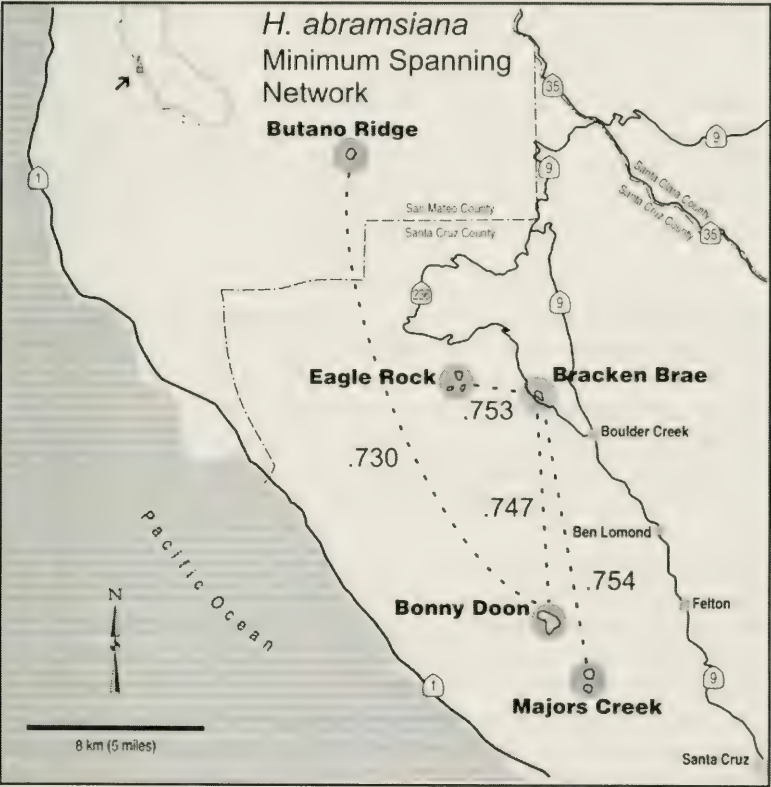


Figure 6. Minimum spanning network (dotted lines) superimposed on a geographic map of *H. abramsiana* groves.

in their ISSRs (Figs. 4-6). None of the subspecies proposed by Silba (2003) are supported by terpenoid or DNA data, except the Butano Ridge grove. This taxon is recognized as a variety:

Hesperocypris abramsiana (C. B. Wolf) Bartel var. *butanoensis* (Silba) Bartel & R. P. Adams, **comb. nov.**

Basionym: *Cupressus abramsiana* C. B. Wolf subsp. *butanoensis* Silba, J. Intl. Conifer Pres. Soc. 10: 34. 2003.

Type: On sandstone slope, Butano Ridge, Santa Cruz mountains, north of Big Basin. (The cypress area includes the common corner of sections 11, 12, 13, 14 of township 8 South, Range 4 West, Mount Diablo Base and Meridian, Santa Cruz Quadrangle.), San Mateo County, 1 Sep 1951, C. McMillan 1620 (with P. McMillan, R. Bacigalupi, L. Heckard and H. Dutton) (holotype - NY).

McMillan (1952) reiterated the above locality the holotype with his description that a "trek of approximately one-quarter mile down the slope leads directly into the cypress area, which probably includes the common corner of sections 11,12,13, and 14, of Township 8 South, Range 4 West, Mount Diablo Base and Meridian, Santa Cruz quadrangle." However, the grove does not occur near this common corner of sections, but rather the cypresses are largely centered at N 37° 14.512', W 122° 15.127' on land managed by Pescadero Creek County Park.

McMillan (1952), in his article reporting the discovery of a third *H. abramsiana* grove, the Butano Ridge grove, noted that a "striking difference among the [then three known] populations is to be found in the size of the female cone." After randomly sampling 100 seed cones from each grove, McMillan (1952) reported that the average length of the Butano Ridge grove was 28 mm, Eagle Rock grove was 24 mm, and Bonny Doon grove was 21 mm. With the exception of the Bracken Brae grove (n=11), we measured 45 to 70 cones per grove to validate and update McMillan's data (Table 1). Not only were seed cones from Butano Ridge consistently longer than the other four groves, but the cones also were consistently wider with only four cones from the other groves falling within the measured range of the Butano Ridge grove cones.

Table 1. Grove-by-grove comparison of *H. abramsiana* seed cones.

	Butano Ridge	Eagle Rock	Bracken Brae	Bonny Doon	Majors Creek
mean cone length (mm)	27.0	22.1	16.7	22.0	19.9
mean cone width (mm)	25.5	20.0	16.1	18.8	18.3
mean number of scale pairs per cone	4.7	4.2	3.6	4.3	4.4

Like cone size, the number of scales per cone pairs varies from cone to cone. McMillan (1952) reported that the Butano Ridge grove averaged 5.2 scale pairs per cone with 5 and 6 pairs “common.” While 48% of our sampled cones had 5 and 6 scale pairs, the remaining cones had 4 pairs per cone. In contrast to Butano Ridge, McMillan (1952) reported that the Bonny Doon and Eagle Rock groves “were predominantly of 8-scaled [4-paired] cones, although the average number for the hundred cones was found to be 8.5 [4.3 pairs] and 8.7 [4.4 pairs] respectively.” However, because we found that the Bonny Doon (31%) and Major Creek (45%) groves often have 5 scale pairs, only cone width and length (especially the former measurement) appear to reliably differentiate the Butano Ridge grove from the four other groves.

McMillan (1952) noted that seed color (dark brown to dull black), presence of glaucous seeds, foliage texture (fine versus coarse), and foliage color (dark green versus yellow green) failed to differentiate any of the then three known groves. With the inclusion of the Bracken Brae and Majors Creek groves, our observations confirm that these morphological characters do not consistently differentiate any of the five groves from one another. While the average number of cotyledons per seedling clearly separated the Butano Ridge and Eagle

Rock groves from the Bonny Doon grove (McMillan 1953), additional work is needed to determine whether this character has any taxonomic merit for *H. abramsiana* groves.

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**FRANGULA BETULIFOLIA AND F. OBOVATA
(RHAMNACEAE)
ARE DISTINCT SPECIES**

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ABSTRACT

Frangula betulifolia var. *obovata* occurs in northern Arizona, Nevada, Utah, and Colorado and is geographically disjunct from var. *betulifolia*, which occurs in southern Arizona, New Mexico, Texas, and northern Mexico. The two taxa are consistently different in leaf shape and texture, and with their genetic isolation, each is appropriately treated at specific rank. A new combination to this effect is made here: ***Frangula obovata* (Kearney & Peebles) Nesom & Sawyer, comb. et stat. nov.** *Phytologia* 91(2): 300-307 (August, 2009).

KEY WORDS: *Frangula betulifolia*, *F. obovata*, *F. ×blumeri* Rhamnaceae, taxonomy.

Frangula (*Rhamnus*) *betulifolia* (Greene) Grubov has been treated without formal variants by Johnston (1971), Johnston and Johnston (1969, 1978), Cronquist et al. (1997), and Welsh et al. (2003). In contrast, *Rhamnus betulifolia* var. *obovata* Kearney & Peebles was described by botanists working in Arizona, the only state where both of the putative varieties occur, and a recent treatment for Arizona maintains them as separate (Hill 2008). Although Cronquist et al. (1997) identified the plants as *R. betulifolia*, the corresponding illustration in his treatment depicts var. *obovata*.

According to Kearney and Peebles (1960, p. 532), "The typical plant [of *Frangula betulifolia*], with elliptic or oblong leaves, is limited in Arizona to the south-central and southern counties. In the

northern part of the state is found var. *obovata* Kearney & Peebles, the type of which was collected on Navajo Mountain, Coconino County. This variety is apparently common in and near the Grand Canyon and Havasu Canyon, and extends into southern Utah and Nevada, thus being well separated geographically from the main area of *R. betulifolia*. The variety is characterized by more or less obovate leaves with thicker, more prominent veins.”

The current study corroborates the observations of Kearney and Peebles. The two taxa are consistently and discontinuously different in leaf morphology. Var. *betulifolia* occurs from northern Mexico into southeastern Arizona, southern New Mexico, and trans-Pecos Texas; var. *obovata* is geographically disjunct and occurs in northern Arizona and adjacent Nevada, Utah, and Colorado (Figs. 1 and 2). In view of their morphological and geographic distinction, recognition of each at specific rank is appropriate.

1. Leaf blades elliptic to oblong, elliptic-ovate, or narrowly ovate, 1.6–2.6(–2.9) times longer than wide, relatively thin or slightly thickened, paler beneath, lateral veins (8–)9–13 pairs
..... **Frangula betulifolia**
1. Leaf blades obovate to oblong-obovate or oblong, 1.2–1.8(–2.5) times longer than wide, distinctly thickened and nearly coriaceous, evenly colored on both surfaces, lateral veins (5–)6–8(–9) pairs.
..... **Frangula obovata**

Frangula betulifolia (Greene) Grubov, Trudy Bot. Inst. Akad. Nauk S.S.S.R., Ser. 1, Fl. Sist. Vyssh. Rast. 8: 268. 1949. *Rhamnus betulifolia* Greene, Pittonia 3: 16. 1896. TYPE: USA. New Mexico. [Catron Co.:] along streams, Mogollon Mountains, 20 Jul 1881, *H.H. Rusby* 63 (holotype: US-digital image!; isotypes: MO!, NY-digital image!).

Shrubs or small trees 1–4 m, unarmed, stems brown to gray-brown, glabrous or pubescent. **Leaves** deciduous, alternate, petioles (2–)5–16 mm, blades elliptic to oblong, elliptic-ovate or narrowly ovate, (4–)4.5–10 cm x (2–)2.5–5.5 cm, 1.6–2.6(–2.9) times longer than wide, thin or thickened but not coriaceous, green above, yellowish and paler beneath, hirtellous to hirsutulous on both surfaces, glabrescent,

lateral veins (8–)9–13 pairs, margins serrate to subcrenate, not revolute, apices acute to obtuse, sometimes slightly acuminate, bases obtuse to truncate or rounded. **Flowers** bisexual, 5-merous, 2–20(–38) in pedunculate axillary fascicles, peduncles (flower) (0–)1–10 mm, pedicels (flower and fruit) 3–7 mm. **Stigmas** 3-lobed. **Drupes** globose, 5–10 mm, black, stones (2–)3(–4).

Flowering Apr–Jun. Cliff bases, ledges, moist canyons, ridges, roadsides, rocky slopes, stream banks, Gambel's oak, oak-pine, pine-walnut-maple, white fir; 900–2750 m. Ariz., N.Mex., Tex.; Mexico (Chihuahua, Coahuila, Durango, Nuevo León, Sonora, Tamaulipas).

Powell (1997) observed that "The leaves of the specimens from the Guadalupe Mountains are smaller and thinner in texture than those of the Davis Mountains population." Such a difference has not been confirmed here among numerous specimens examined from both areas.

Frangula obovata (Kearney & Peebles) Nesom & Sawyer, comb. et stat. nov. *Rhamnus betulifolia* var. *obovata* Kearney & Peebles, J. Wash. Acad. Sci. 29: 486. 1939. *Frangula betulifolia* subsp. *obovata* (Kearney & Peebles) Kartesz & Gandhi, Phytologia 76: 448. 1994. TYPE: USA. Arizona. Coconino Co.: Near Rainbow Lodge, N end of Navajo Mt., 1920 m, 11 Jun 1938, R.H. Peebles 13930 with E.G. Smith (holotype: US-digital image!; isotypes: MO!).

Shrubs 1–2.5 m, unarmed, stems red to brown or gray-brown, glabrous or pubescent. **Leaves** deciduous, alternate, petioles 5–14 mm, blades obovate to oblong-obovate or oblong, (4–)5–9 cm x 3.2–6 cm, 1.2–1.8(–2.5) times longer than wide, distinctly thickened and nearly coriaceous, green and minutely puberulous to hirtellous on both surfaces, glabrescent, lateral veins (5–)6–8(–9) pairs, margins minutely serrate to nearly entire, not revolute, apices obtuse to truncate or rounded, bases truncate to subcordate. **Flowers** bisexual, 5-merous, 2–12 in pedunculate axillary fascicles, peduncles (flower) 3–8(–20) mm, pedicels (flower and fruit) 3–10 mm. **Stigmas** 3-lobed. **Drupes** globose, 5–8 mm, black, stones 3.

Flowering Apr–Jun. Canyon bottoms, cliff faces, stream and creek banks, hanging gardens, talus, seepage below cliffs; 1350–2350 m. Ariz., Colo., Nev., Utah.

Frangula obovata has been collected infrequently in Nevada, but the identity of the plants there is unequivocal. Nevada. Clark Co.: Sheep Range, Grapevine Spring area, *Rhamnus*-white fir, 6300 ft, 19 Sep 1978, *Ackerman 31463* (TEX); Charleston (Spring) Mountains, Kyle Canyon, gravelly side of ravine with *Pinus ponderosa* var. *scopulorum* and *Cercocarpus ledifolius*, 2425 m, 11 Aug 1937, *Clokey 7579* (LL, TEX). Wolf (1938) cited the same Clokey collection as well as one other collection of “*Rhamnus betulifolia*” from Kyle Canyon (24 Jun 1926, *Jaeger s.n.*, CAS).

Other plants from the Charleston Mountains are nearly identical to many of *Frangula californica* var. *ursina* over its wider range, with coriaceous, abaxially whitened leaves with a dense, close tomentum of stellate hairs: Clark Co.: gravelly wash a mile N of Wilson’s Ranch, *Larrea* belt, 1200 m, 13 Jul 1939, *Clokey 8415* (MO-2 sheets, TEX); Excelsior Canyon, 1200 m, 7 Sep 1941, *Clokey 8762* (MO, TEX); La Madre Mts, Willow Spring, 3 May 1988, *Liston & Meury 740-2* (TEX).

Harrington (1954) noted that *Rhamnus betulifolia* “has been reported close to southwestern Colorado [in Utah] and may be growing in that part of the state.” Most listings of this species in Colorado perhaps have been based on Harrington’s inclusion, but recent accounts of the Colorado flora (e.g., Weber & Wittman 1992; Hartman & Nelson 2001; Snow 2007) have not included it. A collection from La Plata County in southwestern Colorado is identified in the forthcoming Four Corners Flora (Spence 2008) as *Frangula betulifolia*, but it is here recognized as *F. obovata*: La Plata Co.: Fort Lewis College on Ft. Lewis hill, 28 Jun 1976, *V. Murray s.n.* (SJNM 2118).

Wolf (1938, p. 78–79) noted that a collection from Cochise Co. in southeastern Arizona named as *Rhamnus blumeri* Greene appears to be a hybrid between *Frangula californica* var. *ursina* and *F. betulifolia*. Both the holotype (US) and an isotype (DS) “have two pieces of material on them: the one is a vegetative branch which is

obviously *R. betulifolia*, the other the material upon which Greene based his species. The latter resembles *R. californica ursina*, but is larger in leaf size and has less pubescence. In 1928, I collected around Paradise and obtained material of *R. betulifolia* (C.B. Wolf 2595). The other collections were made from large bushes resembling *R. californica ursina* in habit but only lightly pubescent on the under surfaces of the leaves. These suggest intermediates between *R. californica ursina* and *R. betulifolia*, but are slightly different from the type of *R. blumeri*: Collections: C.B. Wolf 2592 (RSA), 2593 (RSA).” Study of an isotype of *R. blumeri*, specimens of Wolf 2592 (MO), Wolf 2593 (MO), Wolf 2595 (MO-2 sheets), and Wolf & Everett 11384 (TEX), essentially corroborate Wolf’s observations. From numerous other collections of both species from the Chiricahua Mountains, however, we conclude that if hybridization has taken place between *F. betulifolia* and *F. californica* in Cochise Co., it apparently has not been a common occurrence and there is no evidence at hand of introgression.

Frangula ×blumeri (Greene) Kartesz & Gandhi, Phytologia 76: 448. 1994. *Rhamnus blumeri* Greene, Leaflet Bot. Observ. Crit. 2:266. 1912. TYPE: USA. Arizona. Cochise Co.: Chiricahua Mountains, Paradise, small tree near creek, 5300 ft, 28 Aug 1906, J.C. Blumer 1290 (holotype: US, digital image!; isotypes: DS, MO!).

Johnston (1971) noted that *Frangula betulifolia* is “extremely similar to, and probably conspecific with [*Frangula caroliniana* (Walt.) A. Gray]” of eastern North America. The two taxa are closely similar, but they are allopatric and have never been formally merged into a single species.

ACKNOWLEDGEMENTS

We are grateful to the staffs at MO and TEX for help and hospitality during visits there, to Ken Heil for information on SJNM collections, and to Dr. Billie Turner for his comments on the manuscript.

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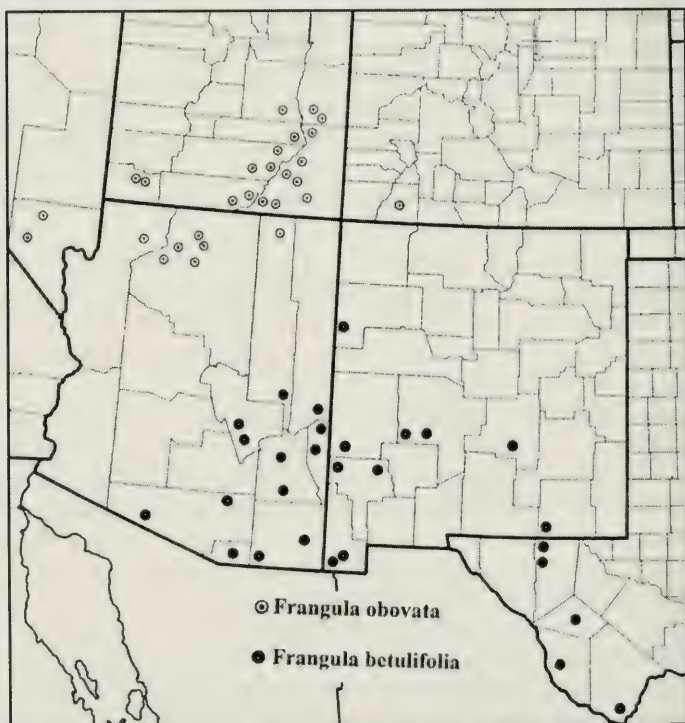


Figure 1. Geographic distribution of *Frangula betulifolia* and *F. obovata* in the U.S.A. Map points are from specimens at LL, MO, SJNM, and TEX, augmented by records from Hill (2008), UVSC Virtual Herbarium (2008), and Albee et al. (1988).

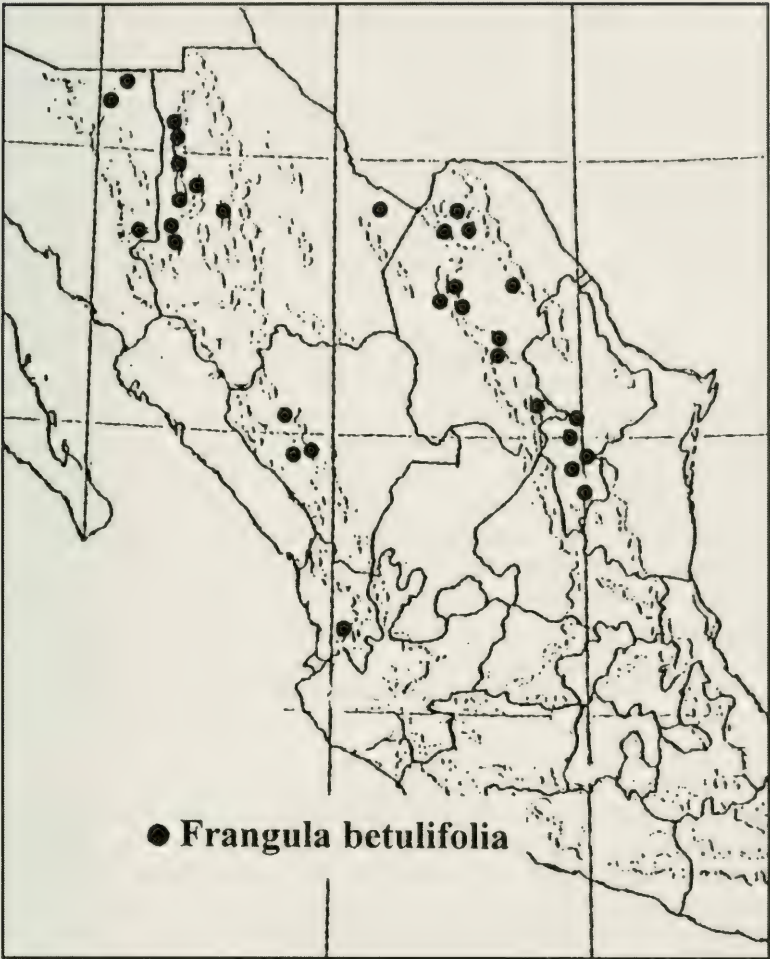


Figure 2. Geographic distribution of *Frangula betulifolia* in Mexico.

**TAXONOMY OF *ASCLEPIAS HIRTELLA* AND
A. LONGIFOLIA (APOCYNACEAE)**

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ABSTRACT

The taxonomy of *Asclepias hirtella* and *A. longifolia* is briefly reviewed. It is concluded that they are best treated as two very distinct varieties: ***A. longifolia* var. *hirtella*** (Pennell) B,L, Turner, **stat. nov.** and *A. longifolia* (Raf.) var. *longifolia*. The two taxa intergrade to a limited extent in southwestern-most Louisiana and closely adjacent Texas. Typical members of var. *longifolia* in Louisiana are confined to the five easternmost counties of that state; all other collections from elsewhere in Louisiana and eastern Texas are essentially typical elements of var. *hirtella*. A distribution map of the complex in North America is provided. *Phytologia* 90(2): 308-311 (August, 2009).

KEY WORDS: Apocynaceae, *Asclepias hirtella*, *A. longifolia*, Texas, Louisiana

The present contribution was occasioned by the appearance of a paper by White (2008) entitled, "*Asclepias hirtella* (Apocynaceae) newly documented for the flora of Texas." In this, he noted that the taxon had not been previously reported for the state of Texas, documenting the occurrence by a collection from Lamar County. He further commented that Turner et al. (2003) had mapped a Lamar Co. collection as *A. longifolia*, which he took to be *A. hirtella*. White fails to report that the latter had been treated as a subspecies of *A. longifolia* by Farmer and Bell (1985). Indeed, the latter workers annotated all of the Texas material on file at TEX as *A. l.* subsp. *hirtella* (Pennell) Farmer & Bell, annotations of which I concur, except for the rank rendered, as discussed below.

It is likely that White's misconception of the names concerned was occasioned by his reliance upon Woodson's (1945) seminal treatment of *Asclepias*. In this, Woodson failed to map (or cite) *A. hirtella* as occurring in Texas, not having seen sheets from the area, most of these assembled after his treatment; however, he did state, "*Asclepias hirtella* and *A. longifolia* are so closely related that they might better be treated as subspecies." Thus, the treatment of Farmer and Bell who, after the study and annotation of numerous specimens from 14 or more herbaria (including LL-TEX), made formal the infraspecific names concerned.

BASIC TAXONOMY

Asclepias longifolia dates back to 1803, typified by plants from the eastern seaboard, "in sylvis Georgiae occidentalibus." *Asclepias hirtella* is typified by material from Jasper County, Missouri, this collected by Pennell himself and first published in 1919 (as *Acerates hirtella*). Since then it has been accounted for by a bevy of workers and is readily distinguished from *A. hirtella* by a number of characters, as well documented by Pennell in his original description. Indeed, *A. hirtella* is easily distinguished by pubescence alone, possessing a spreading (hirtellous) pubescence along the pedicels, *A. longifolia* having an upcurved-appressed pubescence, such vestiture characteristic throughout its range.

My examination of specimens at LL-TEX suggested that the nomenclatural treatment of Farmer and Bell was sound, but I was curious to know the extent of possible intergradation of the two taxa in Louisiana, hence my loan of material from LSU. I was surprised to find that typical elements of *A. longifolia* were to be found only in easternmost Louisiana (Parishes: Livingston, St. Helena, St. Tammany, Tangipohoa and Washington), the remainder referable to *A. hirtella*, at least by the pedicel vestiture mentioned in the above. Among the Louisiana specimens referred to *A. hirtella*, a few in southwestern Louisiana (Natchitoches Parish: Lynch 3884, LSU; Featherman s.n., LSU) and closely adjacent Texas (Jasper Co.: Orzell & Bridges 5678, TEX; Newton Co.: Correll 36544, LL) were found to have an intermediate pedicel vestiture; these are mapped in Fig.1. It is likely

that such plants are a result of ancestral genetic contamination, and not extant hybridization, since I did not find the two taxa coexisting.

Because of the apparent intergradation, however limited, of *A. hirtella* and *A. longifolia* I prefer to treat the two taxa as infraspecific categories, as proposed by Farmer and Bell, but would instead count these as varieties, as argued by Turner and Nesom (2000) and yet others.

***Asclepias longifolia* var. *hirtella* (Pennell) B.L. Turner, stat. nov.**

Based upon *Acerates hirtella* Pennell, Bull. Torrey Bot. Club 46: 184. 1919.

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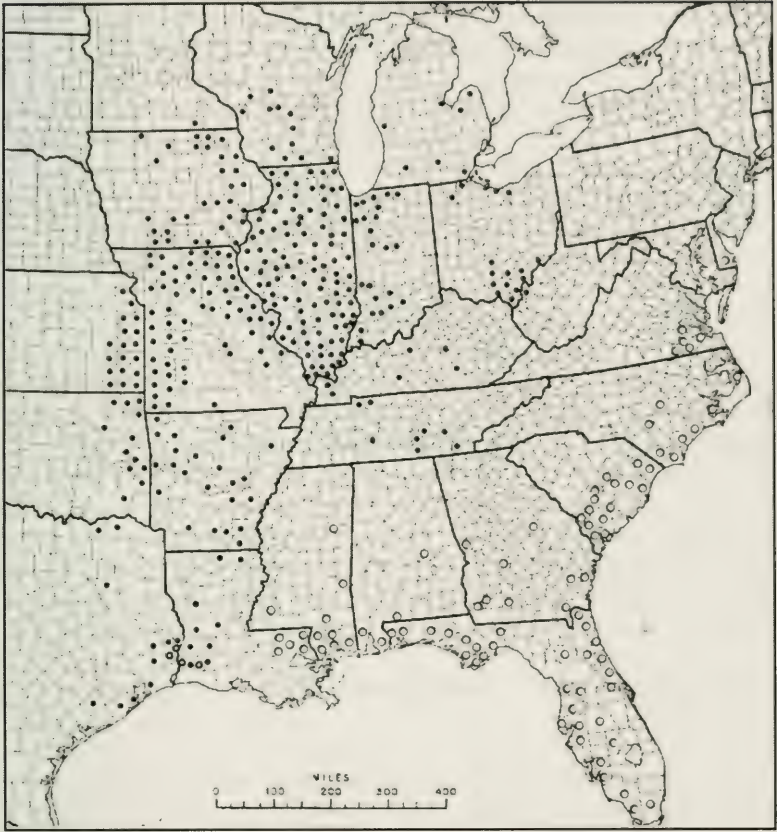


Fig. 1. Distribution of *Asclepias longifolia*: var. *hirtella* (dots); var. *longifolia* (open circles); possible intergrades (small circles).

THREE NEW SPECIES OF *KOANOPHYLLON* (ASTERACEAE: EUPATORIEAE) FROM MEXICO

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ABSTRACT

Three new species of *Koanophyllon* are added to the Mexican Flora: ***K. coixtlahuacum*** B.L. Turner, from Oaxaca; ***K. concordianum*** B.L. Turner, from Sinaloa; and ***K. revealii***, from Guerrero and Oaxaca. In addition, a new varietal combination is proposed: *K. solidaginoides*: var. ***filicaulis*** (Sch.-Bip. ex A. Gray) B.L. Turner, a widespread taxon in eastern Mexico. Distribution maps are provided, along with photoholotypes. The several taxa are keyed along with yet other Mexican taxa in the format of Turner's 1997 treatment of *Koanophyllon* in which 21 species were recognized; the current account brings this total to 24. *Phytologia* 91(2): 312-324 (August, 2009).

KEY WORDS: Asteraceae, Eupatorieae, *Koanophyllon*, Mexico.

Routine identification of Mexican Asteraceae has revealed the following novelties:

KOANOPHYLLON COIXTLAHUACUM B.L. Turner, **sp. nov.**

Fig. 1, Map 1

Koanophyllon richardsonii B.L. Turner similis sed differt laminis foliorum multo majoribus, capitulescentiis magis congestis, et receptaculis glabris (vs pubescentibus).

Shrubs 1-2 m high. **Mid-stems** minutely puberulent to glabrate. **Leaves** opposite throughout; blades broadly deltoid, 6-10 cm long, 4-7 cm wide, 3-nervate from the base, glandular-punctate beneath, sparsely hispidulous above, the margins irregularly serrate; petioles 2.0-3.5 cm

long. **Capitulescence** a terminal congested corymbose panicle of numerous heads, 3-6 cm high, 4-6 cm across, the ultimate peduncles 2-5 mm long. **Heads** ca 7 mm high. **Involucres** ca 4 mm long, composed of ca 11 slender, nearly glabrous, subequal bracts, their apices gradually attenuate. **Receptacles** ca 1 mm across, glabrous or nearly so. **Florets** 13-16 per head; corollas white, glabrous, ca 5 mm long, the lobes ca 0.5 mm long. **Achenes** 2.3-3.0 mm long, markedly hispidulous, especially along the ribs; pappus of ca 40 tawny-white bristles 4-5 mm long.

TYPE: MEXICO. OAXACA: Mpio. Coixtlahuaca, "Concepcion Buena Vista. Km 94.7 de la carretera Tehuacan-Oaxaca (cuota) y de este punto aproximadamente 2 horas a pie montana arriba hasta base de paredes verticales en la cima de cerro." 1680 m, (18 06 58.5 N, 97 19 47.1 W), 27 Oct 1996. *Jose L. Panero & Ismael Calzada 6760* (Holotype: TEX).

The present novelty, in habit and leaf shape, resembles a species of *Fleishmannia*, but it clearly belongs to *Koanophyllon*, where it finds no clear relatives. Panero identified the type as *K. gracilicaule*, which it superficially resembles.

The species is named for the Municipio Coixtlahuaca, from whence the type.

In my account of *Koanophyllon* for Mexico (Turner 1997), I treated all of the latter within a broadly circumscribed *Eupatorium*. I now follow the treatment of King and Robinson (1987). Below find a modified key to the Mexican species of *Koanophyllon*, including the three novelties described herein.

KOANOPHYLLUM CONCORDIANUM B.L. Turner, **sp. nov.**, Fig. 2., **Map 1**

Koanophyllon reyrobinsonii B.L. Turner similes sed differt foliis ovalibus (vs ovatis vel deltoideis ad medium latissimis et flosculis per capitulum paucioribus (4-5 vs 7 vel plures).

Perennial suffruticose herb or sprawling subshrub to 1 m (?) high. **Stems** densely pubescent with mostly upswept hairs. **Leaves** opposite, 5-7 cm long, 3-5 cm wide; petioles 3-6 mm long; blades oval, widest near the middle, 3-nervate from the base, nearly glabrous and atomiferous-glandular below, glabrous above, the margins pubescent, crenulate. **Capitulescence** a terminal corymbose panicle ca 15 cm high, 6-10 cm across, the ultimate peduncles 1-6 mm long, pubescent like the stems. **Involucres** 3-4 mm high, composed of 5-6 subequal, glandular-atomiferous bracts. **Receptacles** ca 0.5 mm across, pubescent. **Corollas** white, atomiferous-glandular, ca 2.5 mm long; tube ca 1.5 mm long; lobes 5, obtuse, ca 0.2 mm long. **Achenes** black, 5-ribbed, ca 1.5 mm long, appressed-pubescent; pappus of ca 30 persistent bristles ca 5 mm long.

TYPE: **MEXICO. SINALOA: Mpio. de Concordia**, "El Capomito, Ejido Los Ciruelos, Comunidad La Guasima," (23 18 10 N, 105 56 12 W), tropical deciduous forest, 341 m, 7 Jan 2006, *A.L. Reina G. et al. 2006-122* (Holotype: TEX).

ADDITIONAL SPECIMEN EXAMINED: **MEXICO. SINALOA: Mpio. Concordia**, "La Cuesta Blanca, la Bajada de Campo Redondo, Comunid La Guasima," ca 13.2 km NE Concordia, 393 m, 25 Nov 2008, *Reina G. 2008-661* (TEX).

This novelty is markedly distinct, having the pubescent receptacles of those taxa centering about *K. longifolium* and *K. reyrobinsonii*, but possessing the capitulescence and heads of *K. palmeri*, to which it is perhaps more closely related.

The species is named for the Municipio de Concordia, from whence the type.

KOANOPHYLLON REVEALII B.L. Turner, *sp. nov.*, Fig. 3, **Map 2**

Koanophyllon gracilicaule (Sch.-Bip. ex B.L. Rob.) King & H. Rob. similes sed differt capitulescentiis minoribus (6-12 mm altis 6-12 mm latis vs ca 20 cm altis 20 cm latis) pedunculis ultimis brevioribus (3-6 mm longis vs 8-15 mm) et setis pappi numerous (ca 40 vs 20).

Shrub or small tree 1-4 m high. **Mid-stems** purplish-brown, minutely hispidulous. **Leaves** opposite throughout; blades ovate to ovate-deltoid, 4-6 cm long, 2-4 cm wide, 3-nervate from the base, glandular-punctate, the margins crenulate; petioles 2-4 cm long. **Capitulescence** a terminal corymbose panicle, 6-12 cm high, 6-12 cm across, the ultimate peduncles hispidulous, mostly 3-6 mm long. **Heads** numerous, 6-7 mm high. **Receptacles** ca 1 mm across, glabrous or nearly so. **Involucres** 3-4 seriate, imbricate, 1-4 mm long, densely brown-hispidulous throughout, linear-lanceolate, their apices abruptly acute. **Florets** ca 15 per head. **Corollas** white, glabrous, ca 4 mm long, the lobes deltoid, ca 0.5 mm long. **Achenes** ca 2 mm long, subglabrous to sparsely hispidulous; pappus of ca 40 tawny persistent bristles 3-4 mm long.

TYPE: MEXICO. GUERRERO: Mpio. Atoyac de Alvarez, "along the Millpillas-Atoyac road via Puerto del Gallo, about 48.5 miles northeast of Atoyac and 6.8 miles southwest of Puerto del Gallo, in mixed deciduous forest with scattered tree ferns on steep slopes," ca 6800 ft, 19 Oct 1975, *J.L. Reveal, K.M. Peterson, R.M. Harley & C.R. Broome 4346* (Holotype: TEX).

ADDITIONAL SPECIMENS EXAMINED: MEXICO:

GUERRERO. Mpio. Atoyac de Alvarez, "below Puerto El Gallo along road to Atoyac." 2255 m, 10 Oct 1986, *Breedlove 65118* (TEX); 38.5 km NE El Paraiso, rumbo a filo de Caballo, 7 Sep 1983, *Villasenor 555* (TEX).

OAXACA: Mpio. Santiago Juxtlahuaca, ca 5 km del poblado El Manzanal, 17 13 0.20 N, 38 04 33.7 W, 22 Aug 1996, *Calzada 21163* (TEX).

In my treatment of the *Koanophyllon* complex for Mexico (Turner 1997) I included the above collections within *K. gracilicaule* (to which it is clearly related). The latter is typified by material from Tlacolula, Oaxaca (GH!), first collected by Ehrenberg in 1839. *Koanophyllon revealii* differs from the latter in having much smaller capitulescences, with shorter ultimate peduncles, somewhat smaller heads, and pappus with more numerous bristles, as noted in the above diagnosis. Distribution of the two taxa is shown in Fig.4.

The species is named for James L. Reveal, Systematist extraordinaire, still kicking up nomenclatural novelties and academic miscellany at the age of eighty. Bravo! May he dance on.

KOANOPHYLLON SOLIDAGINOIDES (H.B.K.) King & H. Rob.,
Phytologia 22: 151. 1972. **Map 3**
Eupatorium solidaginoides H.B.K.

Weak-stemmed, arching or clambering, shrubs 1-3 m high; stems striate, densely puberulent; leaves 6-10 cm long, 2-5 cm wide; petioles mostly 1.5-4.0 cm long; blades deltoid to decidedly cordate, 3(5)-nervate from at or near the base, densely minutely glandular-punctate beneath, glabrous except along the major veins, the margins crenulate to dentate; heads white, numerous in both terminal and axillary, loose or congested, corymbose racemes, the ultimate peduncles 2-10 mm long; florets 10-15 per head; achenes ca 2 mm long, the pappus of 40-50 bristles 2.5-3.0 mm long.

A widespread, highly variable, species but readily distinguished by its weak clambering stems and cordate leaves (rarely deltoid).

Two varieties are recognized in the complex for Mexico, as follows:

Ultimate peduncles 3-7 mm long; heads 5-7 mm high; eastern Mexico.....var. **filicaulis**
Ultimate peduncles 1-3 mm long; heads 4-5 mm high; western Cps.....var. **solidaginoides**

var. filicaulis (A. Gray) B.L. Turner, **comb. & stat. nov.** Map 3.
Koanophyllon solidaginoides **var. filicaulis** (Sch.-Bip. ex A. Gray) B.L. Turner, **comb. & stat. nov.** Based upon *Eupatorium filicaule* Sch.-Bip ex A.Gray, Proc. Amer. Acad. Arts 21: 384. 1886.

San, Ver, Oax, Cps and Guatemala southwards, in barrancas of montane cloud forests 20-2600 m; Nov-Feb.

In Mexico, the two varieties are quite distinct; in Central America, however, they appear to intergrade, especially in northern Guatemala (numerous specimens in and about Tikal, LL-TEX), hence my treatment of these at the varietal level.

var. **solidaginoides** Map 3.

The type of this taxon is from Ecuador. In Mexico, it is known only from Chiapas, the latter populations easily recognized from the typical var. by its much shorter ultimate peduncles and smaller heads. *Eupatorium solidaginoides* var. *armourii* B.L. Rob. (photoholotype FM!) from Palenque, Chiapas appears to be a form of this taxon having markedly deltoid leaves and somewhat larger heads. Additional field studies might show the name concerned worthy of recognition.

Key to Mexican species of *Koanophyllon*

1. Leaves 3-parted or trifoliate on mid-stems (a few leaves simple along the upper stems).....**K. tripartitum**
1. Leaves all simple.....(2)
 2. Leaves pinnately veined.....**K. pittieri**
 2. Leaves with 3-5 principal veins from, or near, the base.....(3)
3. Heads arranged in ball-like clusters, the involucre with only 1 or 2 florets.....**K. monanthum**
3. Heads not as above, the involucre with 4 or more florets.....(4)
 4. Pappus about 0.5 mm long or less; heads in congested, spike-like, capitulescences, the ultimate peduncles mostly 0-1 mm long; Cps.....**K. ravenii**
 4. Pappus 2-7 mm long; heads in mostly open or loosely arranged capitulescences, the ultimate peduncles mostly 2-10 mm long.....(5)

5. Leaves with petioles 1-4 mm long, the blades appearing to clasp;
Ver.....**K. pseudoperfoliatum**
5. Leaves with petioles 3-40 mm long, the blades not appearing to
clasp.....(6)
 6. Petioles mostly 12-40 mm long.....(7)
 6. Petioles mostly 2-15(20) mm long.....(14)
7. Heads 8-10 mm high; achenes densely pubescent with soft
appressed hairs; Baja Calif.....**K. peninsularis**
7. Heads 4-7 mm high; achenes glandular or sparsely hispid; not in
Baja Calif.....(8)
 8. Heads 4-5 mm high.....(12)
 8. Heads 6-8 mm high.....(9)
9. Receptacles glabrous or nearly so; Gue, Oax, Cps.....(11)
9. Receptacles pubescent; Tam to Hid.....(10)
 10. Leaves cordate; achenes hispid; Nue.....**K. hintoniorum**
 10. Leaves ovate, obtuse or truncate at base; achenes to some
degree glandular-pubescent.....**K. richardsonii**
11. Involucral bracts densely pubescent, abruptly
acute.....**K. revealii**
11. Involucral bracts sparsely pubescent, if at all, gradually
attenuate.....**K. coaxtlahuacum**
12. Capitulescence of axillary or terminal spike-like branches, the
heads arranged in tight or loose interrupted corymbs
.....**K. solidaginoides**
12. Capitulescence not as above, terminal and leafy in pyramidal
corymbose panicles.....(13)
13. Blades of leaf pubescent above and below, (rarely glabrate);
corollas 2.5-3.3 mm long; involucral bracts sharply acute;
Chi, Sin.....**K. sinaloense**
13. Blades of leaf glabrous or nearly so; corollas ca 3.5 mm long;
involucral bracts broadly acute; San, Ver, Gue, Oax
.....**K. gracilicaule**

- 14(6). Receptacle glabrous.....(17)
14. Receptacle decidedly pubescent, rarely not; Sin, Nue, Tam, San, Hid.....(15)
15. Leaves oval, widest near the middle; florets 4-5 per head; Sin.....**K. concordianum**
15. Leaves ovate to triangular, widest near the base; florets 6-15 per head.....(16)
16. Blades of leaf uniformly pubescent beneath; petioles mostly 7-15 mm long; corollas ca 2 mm long.....**K. longifolium**
16. Blades of leaf sparsely pubescent along the major veins; petioles mostly 3-6(8) mm long; corollas 2.5-3.0 mm long**K. reyrobinsonii**
17. Suffrutescent herbs or arching, weak, shrubs, 0.5-3.0 m high; older stems not white; foliage to some degree pubescent.....(19)
17. Tree-like shrubs, small trees or woody vines, 2-10 m high; older stems white; foliage glabrous.....(18)
18. Woody vines; capitulescence pyramidal, axillary; leaf blades elliptic, 15-20 cm long; Gue.....**K. guerreroanum**
18. Shrubs or trees; capitulescence corymbose or pyramidal, terminal; leaf blades ovate-elliptic, 6-15 mm long; widespread**K. albicaule**
19. Leaf blades densely pubescent above and beneath; San**K. rzedowskii**
19. Leaf blades not densely pubescent except along the veins beneath; widespread.....(20)
20. Leaf blades variously lanceolate, ovate, or deltoid, but not clearly cordate or subcordate.....(22)
20. Leaf blades neatly cordate or subcordate.....(21)
21. Involucral bracts rigid with narrowly acute (acicular) apices, not at all scarious; Nue.....**K. galeanum**
21. Involucral bracts not rigid, scarious marginally, the apices obtuse or rounded; widespread but not in Nue**K. solidaginoides**

- 22. Leaves often alternate above, the blades epunctate beneath; e Chi, Coa, Dur, Zac.....**K. solidaginifolium**
- 22. Leaves opposite throughout, the blades glandular-punctate beneath; Son, w Chi, Dur, Jal, Col, Mic.....(23)

- 23. Heads 7-9 mm high; Cps.....**K. coulteri**
- 23. Heads 4-6 mm high; w Chi, Sin, Dur, Nay, Jal, Col, Mic, Gue
.....**K. palmeri**

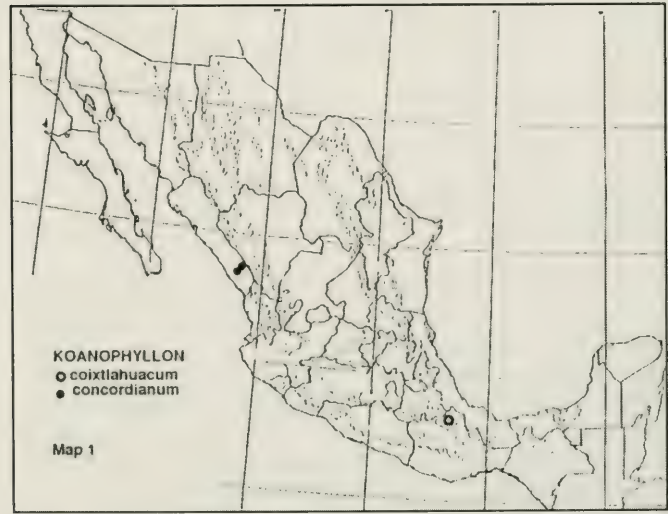
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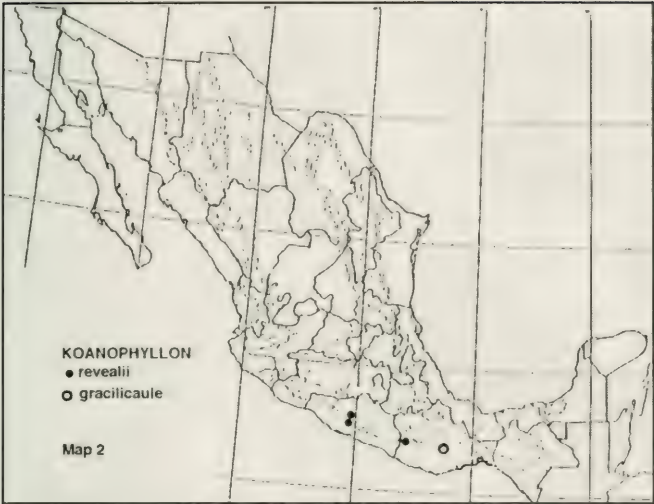
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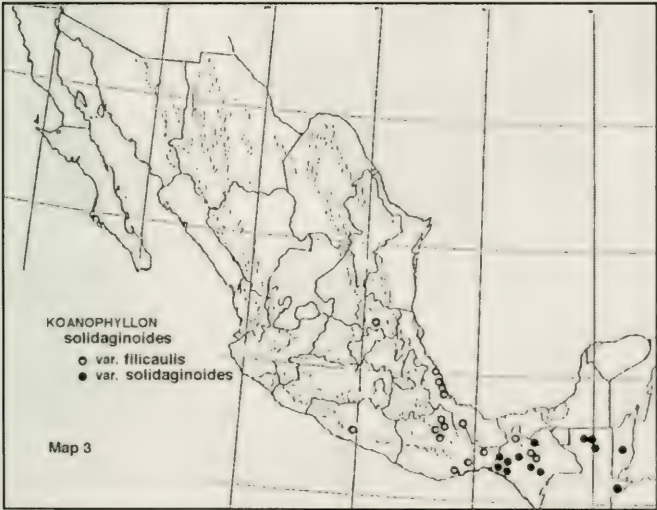
Turner, B.L. 1997. *Koanophyllon*, in The Comps of Mexico. Phytologia Memoirs 11:110-111.



Map 1. Distributions of *K. coixtlahuacum* and *K. concordianum*.



Map 2. Distributions of *K. gracilicaule* and *K. revealii*.



Map 3. Distributions of *K. solidaginoides* var. *filicaulis* and var. *solidaginoides*.



Fig.1. Holotype of *Koanophyllon coixtlahuacum*.



Fig. 2. Holotype of *Koanophyllon concordianum*.

NOTES ON NON-NATIVE ASTERACEAE IN TEXAS

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ABSTRACT

Documentation and/or commentary is provided for 16 of the 73 species of non-native Asteraceae reported to occur outside of cultivation in Texas. *Arctium minus*, *Logfia* (*Filago*) *arvensis*, *Madia elegans*, *Matricaria courrantiana*, *Sanvitalia procumbens*, and *Sonchus arvensis* are removed from the current account of plants naturalized in Texas. *Carduus tenuiflorus* is more appropriately and consistently identified as *C. pycnocephalus*. *Phytologia* 91(2): 325-332 (August, 2009).

KEY WORDS: Texas, flora, adventive species

73 species of non-native Asteraceae have been recorded as occurring outside of cultivation in Texas (Nesom 2009). About 37 of these were reported after publication of the Manual of the Vascular Plants of Texas (Correll & Johnston 1970). The present report provides taxonomic clarification and voucher information for a number of the latter, where ambiguity existed or documentation was lacking.

1. *Arctium minus* Bernh.

Hultén and Fries (1986, Vol. 2, Map 1853) mapped the species for Texas, at the southern extremity of its adventive range in North America, placing a dot near the center of the state. Its occurrence in Texas may be expected, as it is documented to occur in New Mexico, Oklahoma, Arkansas, and Louisiana (USDA, NRCS 2009), but no voucher or other documentation has been located for its occurrence in Texas (Keil 2006a) and the species is removed from the current account of Texas plants.

2. *Carduus acanthoides* L.

Listed by Jones et al. (1997); mapped by Turner et al. (2003) for Collin and Schleicher counties. Vouchers are at TEX.

Collin Co.: Farm Rd 981 just E of jct with Hwy 78, bottomland forest, 13 May 1992, *Saunders 3418* (TEX). **Schleicher Co.:** Westernmost portion of Eldorado, weed along Hwy 190, 8 Jun 2001, *Turner 21-770* (TEX).

3. *Carduus pycnocephalus* L.

Identification and taxonomy of *Carduus pycnocephalus* must be considered in conjunction with that of the very similar *C. tenuiflorus* Curtis. Cory (1940) collected plants of *C. pycnocephalus* in Sutton Co. in 1939, in pastures and fields growing with plants of *Silybum marianum* (L.) Gaertn. and *Carduus nutans* L., all three of which apparently arrived there in a shipment of hay from California. In a later collection from Sutton Co. (*Cory 53662*, 5 May 1947, SMU), he had changed the identification to *C. tenuiflorus*. *Carduus tenuiflorus*, as it has been subsequently identified in Texas, now has spread over a large region of central Texas (Turner et al. 2003).

Dunn (1976) identified the Texas plants as *Carduus pycnocephalus*, and Keil (2006b) included Texas among the states in the known range of *C. pycnocephalus* ("Aggressive weed of waste ground, rangelands, pastures, roadsides, fields; Ala., Ark., Calif., Idaho, Miss., N.Y., Oreg., Pa., S.C., Tex."). He including only California and Oregon in the North American range of *C. tenuiflorus*, noting that it has been reported from New Jersey, Texas, and Washington but that he had not seen specimens from those states.

Comments on the subtle and quantitative distinctions between *Carduus pycnocephalus* and *C. tenuiflorus* were provided by Keil (2006b). Keil also noted that "Published chromosome counts ($2n = 18, 31, 32, 54, 60, 64, 80$) for *C. pycnocephalus* from a variety of Old World localities indicate that this is a complex species in need of further investigation. ... The two species sometimes grow in mixed populations and at times appear to intergrade. Hybridization has been reported in Europe ... and is suspected to occur in California." The two also have been treated as conspecific, the younger name becoming *C. pycnocephalus* L. var. *tenuiflorus* (Curtis) Fiori.

It seems clear that only a single evolutionary entity is represented among the Texas plants, whether from the initial

introduction in Sutton Co. or from multiple introductions. The morphology and variability in Texas are similar to that characteristic of California plants of *Carduus pycnocephalus* as well as all or almost all California collections at BRIT and TEX identified as *C. tenuiflorus*. The Texas plants are more appropriately and consistently identified as *Carduus pycnocephalus* rather than *C. tenuiflorus*.

4. *Carthamus tinctorius* L.

Mapped by Turner et al. (2003) for Brazos and Tarrant counties, evidently based on the following collections.

Brazos Co.: College Station, 8 Apr 2006, *Reed & Reed 3086* (TAMU). **Ellis Co.:** Waxahatchie, in yard near a house along FM 55, not in vicinity of a bird feeder, ca. 7 plants in area 15 feet long, 11 Jun 2002, *Hill et al. s.n.* (BRIT). **Tarrant Co.:** Fort Worth, weed in yard, probably from bird seed, 19 Jun 1996, *Sylvester 2414* (BRIT).

Carthamus lanatus L. is known in Texas from Gillespie, McCulloch, Kimble, Schleicher, and Coryell counties (collections at BRIT, TEX), and Llano, Menard, and Tom Green counties (as additionally mapped by Turner et al. 2003), where it has been found in pastures and gravel pits and along roadsides.

5. *Centaurea benedicta* (L.) L. (= *Cnicus benedictus* L.)

Listed by Jones et al. (1997) and included for Texas by Keil and Ochsmann (2006). The Texas record is based on the following (fide Steve Hatch at TAES): Callahan Co.: "grown in a pot from a wheat field," Mar 1993, *J. Landers s.n.* (TAES). The plant apparently was grown to identify a weed submitted for identification.

6. *Cotula australis* (Sieber ex Spreng.) Hook. f.

Recorded by Johnston (1990) based on plants from the Texas A&M campus, as documented here by collections at TEX; the report by Hatch et al. (1990) probably based on the same or similar collections.

Brazos Co.: College Station, campus of A&M University, lawn weed with *Soliva*, 27 Apr 1970, *Correll & Correll 38493* (LL, TEX); Texas A&M University campus, an Australian weed widely adventive in W hemisphere, the first population known in Texas, 23 Apr 1970, *Johnston s.n.* (TEX).

7. *Leontodon hispidus* L.

Mapped by Turner et al. (2003) for Panola Co. An apparently corresponding voucher at TEX is from adjacent **Harrison Co.**: Marshall, SE corner of yard at Grover Hall, East Texas Baptist College, 16 Jun 1983, *Fleetwood* 6 (TEX).

8. *Leontodon taraxacoides* (Vill.) Mérat

Vuilleumier (1973) included "Texas" in the statement of range for the species; mapped by Turner et al. (2003) from El Paso Co. (as *Leontodon* "*taraxacum*"). A voucher at TEX is from **El Paso Co.**: El Paso, 7400 block of N Mesa, in watered lawn, 12 Aug 1993, *Worthington* 22279 (TEX).

9. *Logfia* (Filago) *arvensis* (L.) Holub

This species has been included by the PLANTS Database (USDA, NRCS 2009) in the Texas flora based on Johnston (1990). Johnston, however, noted only that his description of *Stylocline micropoides* Gray in the Texas Manual (Correll & Johnston 1970) was "wholly inappropriate, [and] instead applies to *Logfia arvensis*." The real *Stylocline micropoides*, which is a native species, is vouchered from El Paso. **El Paso Co.**: NW part of El Paso at small andesite hills 0.3 mi. N of jct Belvedere w/ Westwind, 4200 ft., mostly a creosote bush community with several substrate types and arroyos, 29 Apr 1983, *Worthington* 10130 (TEX, UTEP). The real *Logfia arvensis* has not been observed in Texas (Worthington 1989, 1997), and the species is removed from the current account of Texas plants.

10. *Madia elegans* D. Don ex Lindl.

Turner et al. (2003) mapped the species for Grimes County, evidently based on early collections from Plantersville, a town founded in the 1830's and whose population has fluctuated around 200 since about 1900. The Atchison, Topeka and Santa Fe Railway runs through the town and might have been a source of the seeds, but the label data do not indicate that the plants were naturalized or waived in Plantersville, and it seems most likely that they were garden collections. *Madia elegans* has not been reported from anywhere else outside its native range in California, Nevada, Oregon, and Washington, and the species is removed from the current account of Texas plants.

Grimes Co.: Plantersville, Fall 1927, *Bletsch* 42-77 (TEX); Plantersville, Fall 1927, *Bletsch s.n.* (TEX).

11. *Matricaria courrantiana* DC.

Kearney and Peebles (1960, p. 937) included "Texas" in the description of geographic range for the species, and Arizona and Texas remain the only two states recorded for the occurrence of this species in the USA (fide PLANTS). Although the observation by Kearney and Peebles surely was based on real evidence, until a voucher for the Texas record can be located, the species is removed from the current account of Texas plants.

12. *Sanvitalia angustifolia* A. Gray

Recorded by Johnston (1990), with the following comment: "Found once in the campgrounds of the Chisos Mountains basin, perhaps waifed through automobile dispersal from its native home in Mexico." Based on a TEX collection.

Brewster Co.: Big Bend National Park, Chisos Mts. Basin Campground, at the first campsite on the rt. after the paypost, only two plants seen, 13 Oct 1978, *Henrickson & Bontemps* 12444 (TEX).

13. *Sanvitalia procumbens* Lam.

Noted by Strother (1979) to occur "in trans-Pecos Texas in Chisos Basin (J. Henrickson pers. comm.)," with further comment (Strother 2006, p. 71): "*Sanvitalia procumbens* has been seen only sporadically in the flora; it probably is not a resident." Strother (pers. comm.) comments that for the FNANM treatment, he "saw no supporting specimen(s) for *Sanvitalia procumbens* from California or Texas," and it is highly probable that Henrickson's communication was intended to refer to *S. angustifolia*. *Sanvitalia procumbens* is removed from the current account of Texas plants.

14. *Scorzonera laciniata* L.

Recorded by Johnston (1990) to occur in Randall Co., without other documentation. Collections of the species have been made from three Panhandle counties.

Hutchinson Co.: LAMR, Harbor Bay area, directly W of Fritch on Harbor Bay road, roadside and steep red-sandy slopes with dolomite boulders from caprock, 3190 ft, 22 Apr 2002, *Nesom & O'Kennon*

LAMR 16 (BRIT); LAMR, lawn and roadside of NPS Ranger Station, just W of Sanford-Yake road on short spur, near jet of High Plains Road, 25 Apr 2002, *Nesom & O'Kennon LAMR110* (BRIT); W side of Borger along Hwy 136, grassy areas bordering Wal-Mart parking lot, 28 May 2002, *Nesom & O'Kennon LAMR243* (BRIT). **Lubbock Co.:** Canyon Rd, lower slopes and along the bottom of a "lone profile" canyon, 9 Apr 1990, *Rose s.n.* (LL). **Randall Co.:** Camp Don Herrington Boy Scout Camp on Palo Duro Creek, mixed shrub-grassland community, 24 Apr 1985, *Higgins 15271* (TEX); Canyon, E side of town on vacant lot, 29 May 1988, *Worthington 16584* (TEX).

15. *Solivia anthemifolia* (Juss.) Sweet

Turner et al. (2003) mapped *Soliva anthemifolia* for Bastrop Co. and Leon Co., separately from "*Soliva mutisii*," which is shown with a considerably broader range. *Soliva mutisii* Kunth, however, is a synonym for *S. anthemifolia* (Watson 2006), and in the Flora of Texas Database (<http://www.biosci.utexas.edu/prc/Tex.html>), identifications of TEX/LL vouchers for these records have been corrected to *S. stolonifera* (Brot.) Sweet and *S. anthemifolia* (Juss.) Sweet, respectively. Nueces Co. is added to the two other counties for the distribution of *S. stolonifera*. *Soliva sessilis* Ruiz & Pavón (= *S. pterosperma* [Juss.] Less.) also is commonly and widely naturalized in east Texas.

16. *Sonchus arvensis* L.

Hatch et al. (1990) noted that a previous claim that this species occurs in Texas has not been confirmed. Jones et al. (1997) also listed it, but no voucher has been located and Texas was not included in the range of the species in the FNANM treatment (Hyatt 2006). The species is removed from the current account of Texas plants.

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**THYMOPHYLLA TENUILOBA AND T. WRIGHTII
(ASTERACEAE: TAGETEAE)**

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ABSTRACT

Thymophylla tenuiloba has generally been treated to include four varieties. Among these, *T. tenuiloba* var. *wrightii* (A. Gray) Strother is distinct in morphology and sympatric and non-intergrading with *T. tenuiloba* var. *tenuiloba*. It is appropriately treated at specific rank as *T. wrightii* (A. Gray) Small. *Thymophylla tenuiloba* var. *treculii* and var. *texana* differ from var. *tenuiloba* in minor features of pappus morphology and populational variation occurs in the same features; each is geographically distinct, however, and these three taxa are maintained at varietal rank. *Phytologia* 91(2): 333-339 (August, 2009).

KEY WORDS: *Thymophylla tenuiloba*, *Thymophylla wrightii*, Asteraceae, Tageteae, Texas

In Johnston's taxonomic overview of Texas *Dyssodia* (1956), he noted that *D. tenuiloba* (DC.) B.L. Rob., *D. wrightii* (A. Gray) B.L. Rob., *D. texana* Cory, and *D. treculii* (A. Gray) B.L. Rob. "are more closely related to each other than to other species. In details of involucre they are nearly identical; they differ in pappus-form, and to some extent in habit." Strother (1969) emphasized the similarities among these four taxa by combining them as varieties of a single species, *D. tenuiloba*. And so they have been treated since that time (Strother 1970, 2006), except for Turner (1996) and Turner et al. (2003), who combined var. *treculii* and var. *texana* with var. *tenuiloba*, simplifying the species to var. *tenuiloba* and var. *wrightii*. Robinson's treatment (1913) of *D. wrightii* and *D. treculii* at specific rank reflected his general transfer of names from *Hymenatherum* Cass. to *Dyssodia* Cav. rather than a refinement of species concepts. Strother's transfer

(1986) of all these taxa to *Thymophylla* Lag. has been supported by molecular evidence (Loockerman et al. 2003).

In contrast to the generally accepted taxonomy, there is good evidence to treat var. *wrightii* at specific rank. As documented by Strother (1969) and as confirmed here, var. *tenuiloba* and var. *wrightii* are sympatric (Fig. 1), and I find no evidence of hybridization where the two occur together. The two taxa also differ slightly in pappus morphology (see key below) and they are consistently different in leaf morphology. Var. *wrightii* has entire, mostly linear leaves while var. *tenuiloba* has pinnatisect leaves, and there is no indication that toothing or lobing appearing rarely on proximal leaves of var. *wrightii* results from gene flow from var. *tenuiloba*.

The two have been collected at the same site: Karnes Co.: 12 mi S of [Wilson] county line on Texas Hwy 80, 15 Apr 1965, *Strother 137* (TEX)—*Thymophylla wrightii* and *Strother 138* (TEX)—*T. tenuiloba*. Refugio Co.: 18 mi S of Woodsboro, 10 Apr 1965, *Strother 128* (TEX)—*Thymophylla wrightii* and *Strother 129* (TEX)—*T. tenuiloba*. At both localities, Strother identified the plants as different species when he made the collections; he later treated the two taxa at varietal rank. The two also have been collected at very close though not identical localities within their area of sympatry (e.g., Bee Co., San Patricio Co.; Fig. 1).

Chromosome number reports for *Thymophylla wrightii* all have been diploid, $2n = 16$ (Strother 1989). Plants of *T. tenuiloba* var. *tenuiloba* may be diploid, triploid, or tetraploid ($2n = 16, 24$, or 32), and populations may include a single ploidy level or mixtures of two or three ploidy levels. One population of var. *tenuiloba* from Webb Co. was observed to include diploids, triploids, and pentaploids. In the region of sympatry with *T. wrightii*, populations of *T. tenuiloba* are diploid or triploid or a mixture of the two levels (Strother 1989, Fig. 2). Circumstantial evidence indicates that triploids and perhaps other polyploids produce seeds apomictically. Knowledge of this aspect of biology strengthens the observation that *T. wrightii* and *T. tenuiloba* are genetically isolated where they occur in sympatry.

Status of *Thymophylla tenuiloba* var. *texana* and var. *treculii*.

In contrast to *Thymophylla wrightii*, *T. tenuiloba* var. *treculii* and *T. tenuiloba* var. *texana* differ from typical *T. tenuiloba* only in minor features of pappus morphology, and intergradation and populational variation occurs in the same features. Var. *treculii* and var. *texana* are geographically distinct (Figs. 1 and 2), however, and are appropriately treated at infraspecific rank within *T. tenuiloba*. Limited sampling indicates var. *texana* to be diploid, var. *treculii* to be tetraploid and pentaploid (Strother 1989).

Almost all collections of var. *treculii* in Texas have been made very close to the Rio Grande, where it occurs in close sympatry with var. *tenuiloba*. In its broader range in Coahuila and Nuevo León, var. *treculii* occurs alone, thus the area of sympatry along the Rio Grande is where the ranges of var. *treculii* and var. *tenuiloba* meet (Strother 1969, Fig. 19). Mixed populations and intergrades appear to be common in the area of sympatry, although most plants display one or the other of the pappus expressions. Northern outliers of var. *treculii* in Crockett, Sutton, and Uvalde counties may be relatively recent adventives dispersed along roadways, as hypothesized by Strother (1989) for var. *tenuiloba*.

Var. *texana* is rare in Texas and restricted to a few west-central counties, far disjunct from Mexican populations in referable to this entity (Strother 1969, Fig. 20). It apparently does not intergrade with other expressions of *Thymophylla tenuiloba* in Texas, but in Coahuila, plants technically referable to var. *texana* do apparently intergrade with var. *treculii*.

Taxonomic overview.

Morphological criteria for recognizing these taxa, as in the key below, are similar to those of Strother (2006).

1. Plants erect to ascending; **leaves** relatively lax, entire, oblong-linear to filiform, rarely those on the proximal 1/6–1/2 of stem with 2–4 pairs of linear teeth or lobes; **pappus** of 10–12 unequal pales 2–3 mm long, each terminating in a single bristle-like awn or (less commonly) pales of outer series bifid and terminating in a single bristle-like awn; Texas.

Thymophylla wrightii

1. Plants usually diffusely spreading to decumbent, sometimes erect; leaves rigid, pinnatisect into 7–11 subulate, filiform divisions; pappus variable.....**Thymophylla tenuiloba**

2a. Pappus of 10 pales, each 3–5-awned; Texas and Mexico (Tamaulipas).....**T. tenuiloba** var. **tenuiloba**

2b. Pappus of 10 pales in two series of 5 each, those of the inner series 2.5–3 mm long and each 1-awned from the middle of the often bifid apex, those of the outer series 0.8–1 mm long and awnless; Texas and Mexico (Coahuila, Nuevo León, Tamaulipas).**T. tenuiloba** var. **treculii**

2c. Pappus of 10 pales of subequal length in two series of 5 each, all awnless, or the inner 5 slightly longer and occasionally 1 or 2 of them 1-awned; Texas and Mexico (Coahuila).....**T. tenuiloba** var. **texana**

1. **Thymophylla wrightii** (A. Gray) Small, Fl. S.E. U.S., 1295, 1341. 1903. *Hymenatherum wrightii* A. Gray, Mem. Amer. Acad. Arts, n.s, 4(1): 89. 1849. *Dyssodia wrightii* (A. Gray) B.L. Rob., Proc. Amer. Acad. Arts 49: 508. 1913. *Dyssodia tenuiloba* var. *wrightii* (A. Gray) Strother, Univ. Calif. Publ. Bot. 48: 76. 1969. *Thymophylla tenuiloba* var. *wrightii* (A. Gray) Strother, Sida 11: 378. 1986. **TYPE:** USA. Texas. In dry post oak woods between the Rio Colorado and the Rio Guadalupe, *C. Wright* s.n. (holotype: GH).

2. **Thymophylla tenuiloba** (DC.) Small, Fl. S.E. U.S., 1295, 1341. 1903. *Hymenatherum tenuilobum* DC., Prodr. 5: 642. 1836. *Dyssodia tenuiloba* (DC.) B.L. Rob., Proc. Amer. Acad. Arts 49: 508. 1913. **TYPE:** USA. Texas. "In Mexico circa Bejar," [between Laredo and San Antonio], *Berlandier* 2063 (holotype: G-DC; isotype: GH).

2a. **Thymophylla tenuiloba** var. **tenuiloba**

- 2b. **Thymophylla tenuiloba** var. **texana** (Cory) Strother, Sida 11: 378. 1986. *Dyssodia texana* Cory, Rhodora 49: 162. 1947. *Dyssodia tenuiloba* var. *texana* (Cory) Strother, Univ. Calif. Publ. Bot. 48: 76. 1969. **TYPE:** USA. Texas. Taylor Co.: Abilene, Camp Barkeley, grassland in stony clay soil, 26 Apr 1943, *W.L. Tolstead* 7030 (holotype: GH; isotype: SMU!).
- 2c. **Thymophylla tenuiloba** var. **treculii** (A. Gray) Strother, Sida 11: 378. 1986. *Hymenatherum treculii* A. Gray, Proc. Amer. Acad. Arts 19: 42. 1883. *Dyssodia treculii* (A. Gray) B.L. Rob., Proc. Amer. Acad. Arts 49: 508. 1913. *Dyssodia tenuiloba* var. *treculii* (A. Gray) Strother, Univ. Calif. Publ. Bot. 48: 75. 1969. **TYPE:** USA. Texas. "SE Texas [near Eagle Pass], *A. Trecul* s.n. (holotype: GH).

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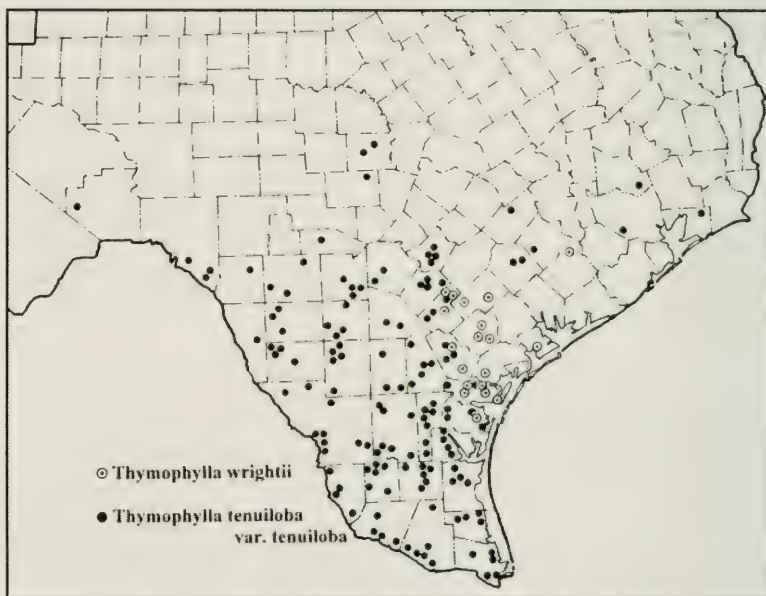


Figure 1. Distribution of *Thymophylla wrightii* and *T. tenuiloba* var. *tenuiloba* in Texas. Collections were first mapped on a large-scale highway map, then transferred to this format (also for Fig. 2). Var. *tenuiloba* ranges into Mexico; it also occurs as an adventive in the U.S.A. in Alabama, California, and Louisiana.

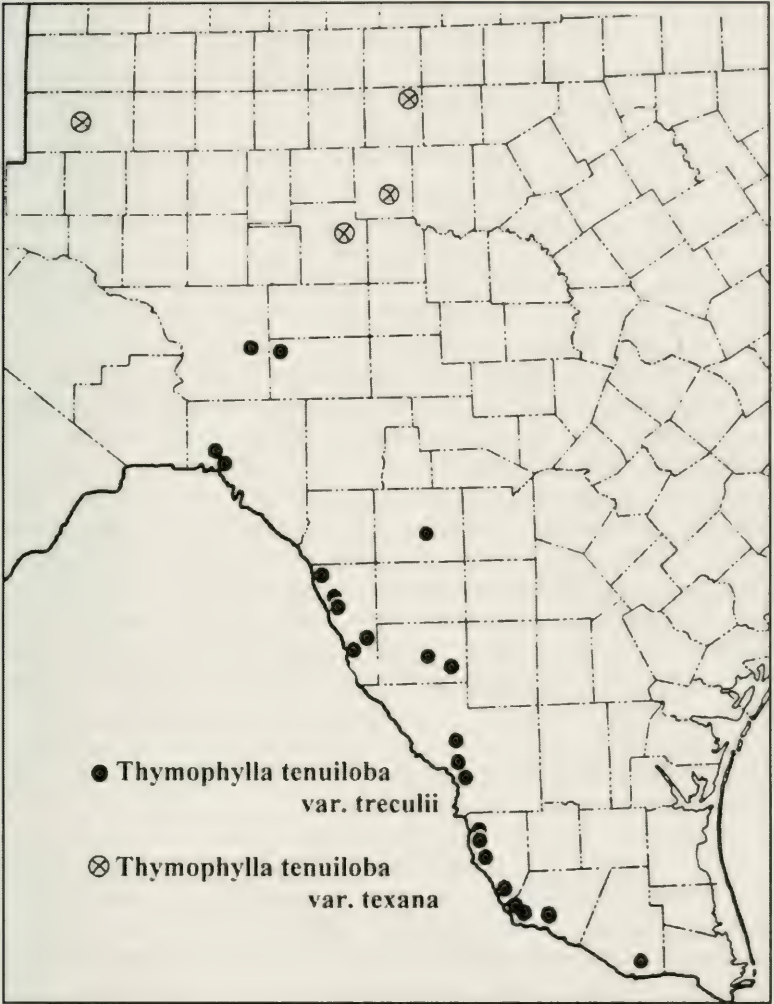


Figure 2. Distribution of *Thymophylla tenuiloba* var. *treculii* and var. *texana* in Texas.

BIOLOGICAL STATUS OF THE VARIETAL TAXA OF
THYMOPHYLLA PENTACHAETA
(ASTERACEAE: TAGETEAE)

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ABSTRACT

The biological status of *Thymophylla* [*Dyssodia*] *pentachaeta* is evaluated; the species was treated by Strother (1969, 1986) as having four infraspecific taxa: var. *belenidium*, var. **hartwegii**, var. **pentachaeta** and var. **puberula**. After examining most of the specimens that his taxonomy was based upon, it is concluded that the all of the taxa are worthy of specific rank, except for var. *belenidium*, which is treated as a synonym of *T. pentachaeta*. Since all of these taxa were previously treated as species, no new names are required. Reasons for the dispositions are given, along with maps showing their distributions, these based upon numerous specimens assembled since the seminal study of Strother. *Phytologia* 91(2): 340-346 (August, 2009).

KEY WORDS: Asteraceae, Tageteae, *Dyssodia*, *Thymophylla*, *T. pentachaeta*, Argentina, Mexico, U.S.A.

Strother (2006) provided a systematic treatment of *Thymophylla pentachaeta* (DC.) Small for the Flora of North America, this largely based upon his doctoral study of the genus *Dyssodia* (s.l.). In this, he recognized a subsp. *hartwegii*, this having but a single var. *hartwegii* (A. Gray) Strother; and a subsp. *pentachaeta*, this having three varieties: var. *belenidium* (DC.) Strother, var. *pentachaeta* (DC.) Small and var. *puberula* (Rydb.) Strother.

In my treatment of the Comps of Mexico (Turner 1996) I inappropriately recognized *Thymophylla pentachaeta* as possessing

only two varieties: var. *pentachaeta* and var. *hartwegii*. My detailed reexamination of the group has led to the present treatment in which *T. puberula* is resurrected, leading to the recognition of three species in the complex. The biological status of each of these is discussed below.

var. *belenidium*

The type of this taxon is from Argentina, based upon specimens obtained in the province of Mendoza by Arnott prior to 1838, the year of its publication. Strother accepted the taxon as a valid variety, and assumed it to be confined to Argentina and the southwestern U.S.A. and adjacent northern Mexico. In his key to taxa, he distinguished the variety from var. *pentachaeta* by its shorter peduncles (2-5 cm vs. longer); outer phyllaries nearly free to the base (vs. not so), these bearing 3-6 pairs of marginal glands (vs. "fewer glands"). If one applies such key leads to specimens from Argentina (on file at LL-TEX) it will be found that both var. *belenidium* and var. *pentachaeta* occur in that country, but such is not noted by Strother. Presumably, he believed Argentina to lack specimens referable to var. *pentachaeta*. Further, I found so much variation in the characters called to the fore by Strother, that I was unable to map a coherent var. *belenidium* in either Argentina or North America. In short, I take the two taxa to be synonymous. Fig. 1 shows the distribution of the two taxa as interpreted by Strother. I would map these as but a continuous, highly variable, var. *pentachaeta*, both in Argentina and North America (Figs. 1, 2 and 3).

var. *pentachaeta* = *Thymophylla pentachaeta* Figs. 3, 5

The type of this taxon is from the state of Nuevo Leon, Mexico, first collected by Berlandier in the vicinity of Monterrey prior to its publication by De Candolle in 1836. As indicated in the above account, I consider *T. belenidium* to be synonymous with var. *pentachaeta*, the characters separating these are highly variable and when mapped as a syndrome do not stand up to meaningful morphogeographical interpretations.

var. puberula = Thymophylla puberula Fig. 4

The type of this taxon is from the state of San Luis Potosi, Mexico, first collected by Schaffer in 1877 in the Valley of San Luis Potosi. As indicated by Strother (his Fig. 18), this taxon is sympatric with *T. pentachaeta* over many a mile of Mexico (Fig. 4). In spite of the numerous populations sampled, very few intermediates between the two taxa have been detected in the field or in the herbarium, either by Strother (at least by annotations on specimens) or myself, this in spite of the fact they often grow in close proximity. Indeed, numerous specimens assembled since Strother's study has shown the two taxa to be easily recognized, intermediates being conspicuously absent, suggesting specific status for both.

var. hartwegii = Thymophylla hartwegii Fig. 6

This species is easily recognized by the characters called to the fore by Strother, hence its treatment as a monotypic subspecies by the latter author. It is known to grow with or near both *T. puberula* and *T. pentachaeta* without the propensity to form recognizable hybrids with either. For example, in Cochise Co., Arizona *T. hartwegii* is said by Barr (63-130, TEX)) to occur "as [a] distinct population but adjacent to *Dyssodia pentachaeta*." In short, it appears to be a good biological species.

A complete synonymy for all of the above taxa is given by Strother (1969).

ACKNOWLEDGEMENTS

I am grateful to my colleagues, Guy Nesom and Jana Kos, for reading the paper and offering helpful suggestions.

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Fig. 1. Bicentric distribution of *Thymophylla pentachaeta*.



Fig. 2. Distribution of *Thymophylla pentachaeta* in Argentina, by Provinces (data from <http://www.tropicos.org>).

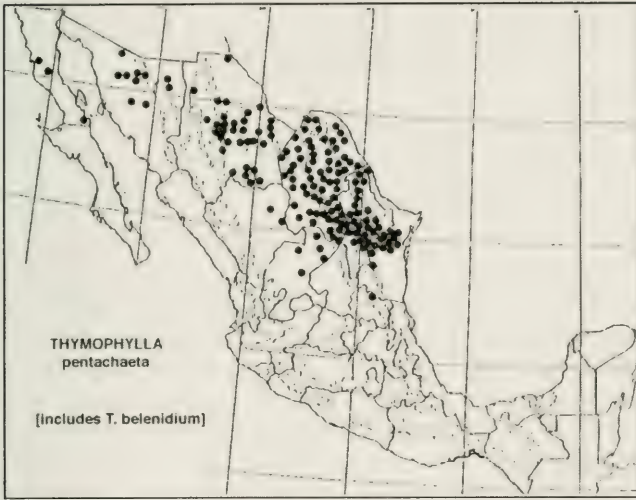


Fig. 3. Distribution of *Thymophylla pentachaeta* in Mexico, as envisioned by Turner (present account).

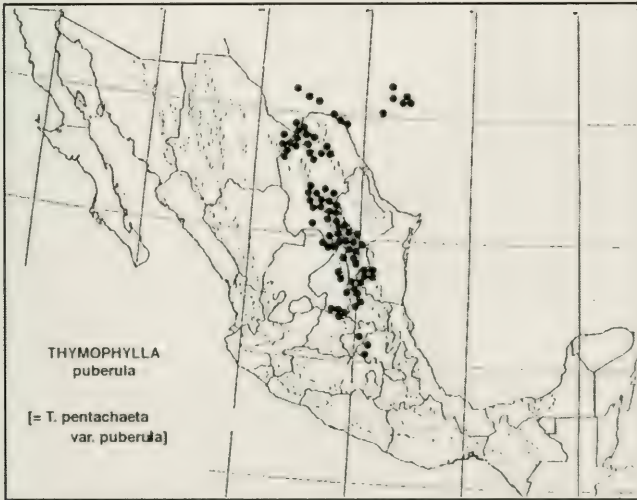


Fig 4. Distribution of *Thymophylla puberula* in North America as envisioned by Turner (present account).

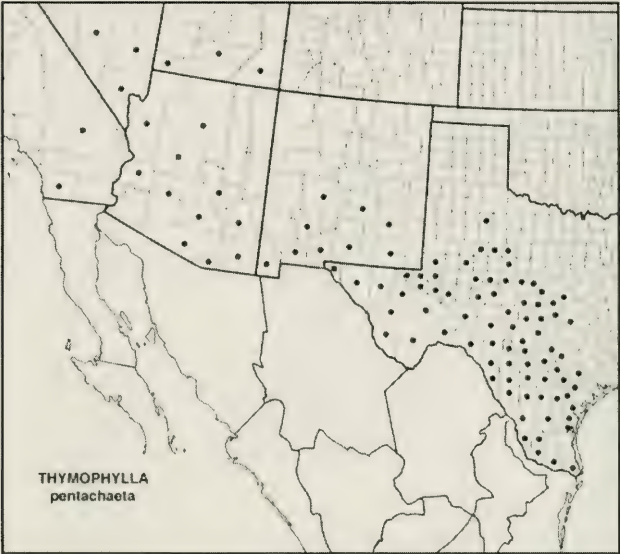


Fig. 5. Distribution of *Thymophylla pentachaeta* in the USA as envisioned by Turner (present account).

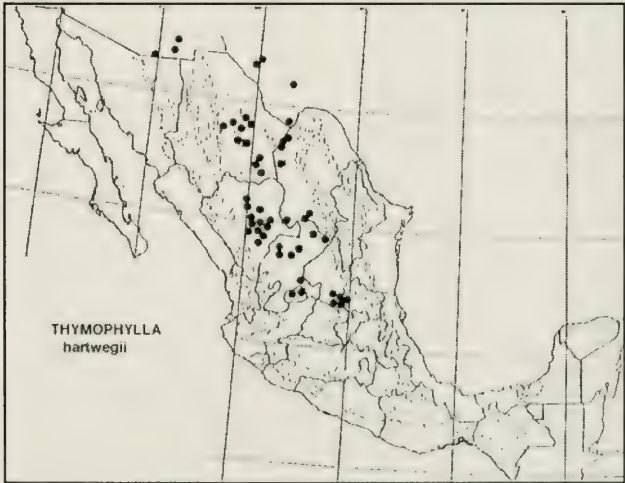


Fig. 6. Distribution of *Thymophylla hartwegii*.

**PANICUM COLORATUM NEW FOR ARIZONA,
AND ECHINOCHLOA HOLCIFORMIS NEW FOR THE
UNITED STATES**

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ABSTRACT

Two new records for Arizona, one of which is new to the United States, are reported. The introduced *Panicum coloratum* L. has previously been found in neighboring states, and is now known from one location in southern Arizona. *Echinochloa holciformis* (H.B.K.) Chase is known from several Mexican states but has not formerly been documented north of the international border. *Phytologia* 91(2): 347-352 (August, 2009).

KEY WORDS: *Panicum coloratum*, Kleingrass, *Echinochloa holciformis*, Poaceae, Arizona.

Author's note: John Raymond Reeder (1914-2009) had been planning a note about these two new records when, in January 2008, he and his wife, Charlotte, were seriously injured in a car accident. Sadly, John never fully regained his former vigor. I am happy to have collaborated with him in the field and herbarium, and to have been included in discussions of these two records. I have prepared this brief communication, at long last, as yet another of his contributions to the grass flora of North America.—KM

Introduced from Africa, the perennial *Panicum coloratum* L. was first documented in Texas from nursery collections in the 1940s and 1950s, and has more recently been collected in uncultivated situations in Texas (Flora of Texas Database, www.biosci.utexas.edu/prc/Tex.html, Feb 2009) and in New Mexico (New Mexico Biodiversity Collections Consortium, nmbiodiversity.org, Feb 2009). The plant, commonly called Kleingrass, was grown in the Soil

Conservation Service Nursery in Tucson, Arizona, in the 1940s, and has been collected in Sonora, México, just south of the border with Arizona (University of Arizona Herbarium Database, ag.arizona.edu/herbarium/search, Feb 2009). The collection reported here is believed to be the first record of the grass outside of cultivation in the state of Arizona. Although favoring the tropics and subtropics, and often found in wet ground (Freckmann and Lelong 2003), occurrences in the southwest region tend to be in ruderal settings but range in elevation up to 1600 m (5250 ft) above sea level.

SPECIMEN: USA. ARIZONA. Cochise County: Parker Canyon Lake parking area, surrounded by vegetation of oaks, junipers, etc. Several clumps, this specimen from one ca. 80 cm diam.; the plants to 1 m tall. Elev. 1600 m. 22 Aug 2002, J.R. Reeder 9846 & C.G. Reeder (ARIZ, US) (Fig. 1).

The New World native *Echinochloa holciformis* (H.B.K.) Chase habitually grows in saturated substrates, often in shallow water and standing a meter or more tall. It is known from several Mexican states including Aguascalientes, Durango, Guanajuato, Jalisco, México, Michoacán, Nayarit, and Puebla, as well as south to Guatemala (McVaugh 1983; Rzedowski and Rzedowski 2001). The nearest occurrence to Arizona documented by herbarium specimens comes from neighboring Sonora, México (A.L. Reina-G. 98-1371, 19 Sep 1998, ARIZ 349502!), about 325 km (203 mi) south of the international border. Since *E. holciformis* was first found in Arizona in 2002, several more collections have been made of this species from three localities, all in southern Santa Cruz County; these are, to-date, the only known records for the United States.

Echinochloa holciformis is distinguished from congeners, particularly *E. polystachya* (H.B.K.) Hitchc. and *E. oplismenoides* (E. Fourn.) Hitchc. that are both known from the southern continental United States (Michael 2003), by the combination of glabrous culms, a conspicuously hairy ligule, an empty sterile (lower) floret, and awn of the sterile lemma ranging 3.5-5.0 cm long (see McVaugh 1983). Gould (1975: 533) reported a specimen from Texas, cited as "Williams in June, 1950 (US)" of which he wrote, "appears to be *E. holciformis* (H.B.K.) Chase. This is similar to *E. polystachya* in being a perennial

with well-developed, hairy ligules and large spikelets, and with stamens in the lower floret. ... The specimen from Jefferson County, Texas, has puberulent culm nodes and awns to 20 mm long." Although we failed to locate this specimen, it seems apparent from Gould's description that the plant represented was, in fact, consistent with *E. polystachya*, and was not *E. holciformis*. The annual species *E. oplismenoides* was, like the records of *E. holciformis* reported here, first documented in the United States by a collection from southern Arizona (Fishbein 1995); although broadly sympatric, it is readily distinguished from *E. holciformis* by a glabrous ligule and shorter awns.

Echinochloa holciformis is regarded as a perennial in its southern range (McVaugh 1983; Rzedowski and Rzedowski 2001), however the plants in the southern Arizona populations are clearly annual, with individuals dying completely to the substrate in the fall and populations varying greatly in size from year to year. The plants typically grow in shallow water, and often develop stout, fibrous roots from the lower nodes, but we have not observed structures that could be interpreted as creeping stems or rhizomes among the populations reported here.

SPECIMENS: USA. ARIZONA. Santa Cruz County: **Canelo Hills**, ca. 1 km N of Canelo Pass summit, rocky slope in oak-juniper area. Growing thickly on the margin of a cattle tank full of water. Elev. 1650 m. 10 Oct 2002, J.R. Reeder 9894 & C.G. Reeder (ARIZ). **Canelo Hills**, ca. 1 km S of trail head to Arizona Trail along FS-799. Around a cattle tank a short distance W of the road, base of plants in the water. Elev. 1550 m. 7 Sep 2004, J.R. Reeder 10002 & C.G. Reeder (ARIZ); 7 Sep 2004, J.R. Reeder 10003 & C.G. Reeder (ARIZ); 22 Sep 2004, J.R. Reeder 10006 & C.G. Reeder (ARIZ, MO, US); 20 Sep 2005, J.R. Reeder 10020 & C.G. Reeder (ARIZ, CAS, MO, NMC, TEX, US). **San Rafael Valley**, just S of entrance to Little Outfit Ranch. Grassland with a few scattered oaks and junipers. On margin of cattle tank a short distance from the road. Elev. 1550 m. 20 Sep 2005, J.R. Reeder 10021 & C.G. Reeder (ARIZ, US); 15 Sep 2006, J.R. Reeder 10028 & K. Mauz (ARIZ, MO) (Fig. 2).

ACKNOWLEDGEMENTS

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Fig. 1. *Panicum coloratum* L. Near Parker Canyon Lake, Cochise County, Arizona, 22 Aug 2002 (ARIZ).



Fig. 2. *Echinochloa holciformis* (H.B.K.) Chase. San Rafael Valley, Santa Cruz County, Arizona, 15 Sep 2006 (ARIZ).

**VARIATION IN *JUNIPERUS DURANGENSIS* AND RELATED
JUNIPERS (CUPRESSACEAE):
ANALYSIS OF nrDNA AND petN SNPs**

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ABSTRACT

Recent discovery of a low shrub from Topia, in the state of Durango, Mexico that appears similar to both *J. durangensis* and *J. jaliscana*, prompted the analyses of nrDNA and petN-psbM (cpDNA) SNPs. The plants from Topia differed from *J. durangensis* by 2 indels but were shown to be closely related as shown in a minimum spanning network. *Phytologia* 91(2): 353-358 (August, 2009).

KEY WORDS: *Juniperus durangensis*, *J. monticola*, *J. martinezii*, *J. flaccida*, nrDNA, petN-psbM, SNPs, Cupressaceae, geographic variation.

Juniperus durangensis Mart. is a tree or large shrub to 5 m that generally branches near the base (Adams, 2008). It is often found on rhyolite, a nutrient poor rocky volcanic substrate, in the mountains of western Mexico from Sonora and Chihuahua southward to Aguascalientes. *Juniperus durangensis* is in the serrate leaf margined junipers and appears most closely related to *J. martinezii* Perez de la Rosa and then to *J. flaccida* Schlecht, *J. jaliscana* Mart. and *J. monticola* Mart. (Fig. 1).

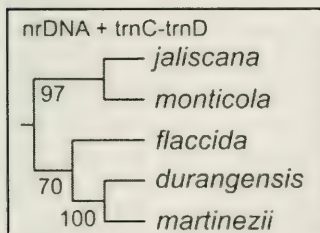


Figure 1. Clade from the serrate leaf margined junipers, based on nrDNA + trnC - trnD (cpDNA) data from Adams (2008) showing the putative relationship of *J. durangensis* to closely related junipers.

Recently, a low growing shrub was discovered near Topia, Durango that seems to be related to *J. durangensis*, although it has some characteristics of *J. jaliscana*. To further investigate the Topia juniper, sequencing of nrDNA and the petN-spacer-psbM cp DNA region were performed to obtain SNPs to reexamine the relationship of the Topia juniper to *J. durangensis* and other closely related junipers.

MATERIALS AND METHODS

Specimens collected (GenBank #: nrDNA; petN-psbM): *J. durangensis*, Adams 6832-6834, (FJ948469, FJ948473) 52 km w of El Salto, on Mex 40, Durango, MX; Adams 11420-11421, (FJ948469, FJ948473)Topia, Durango, MX; *J. flaccida*, Adams 6892-6893, (FJ948470, FJ948476), on Mex. 60, 19 km E. of San Roberto Junction, Nuevo Leon, Mexico; *J. jaliscana*, Adams 6846-6848, (FJ948466, FJ948475), 19 km E of Mex. 200 on the road to Cuale, Jalisco, Mexico; *J. martinezii*, Adams 5950, 5951, 8709, (FJ948471, FJ948474)10 km s of Mex 85 on road to La Quebrada Ranch, Jalisco, MX; *J. monticola* f. *monticola*, Adams 6874-6878, (FJ948467, FJ746736) 1 km n of Mex 105, 9 km nw of Pachuca, El Chico National Park, Hidalgo, Mexico. Voucher specimens are deposited at BAYLU.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia CA).

SNPs obtained from DNA sequencing

ITS (nrDNA) and trnC-trnD amplifications were performed in 50 µl reactions using 10 ng of genomic DNA, 3 units Qiagen Taq polymerase, 5 µl 10x buffer (final concentration: 50 mM KCl, 10 mM Tris-HCl (pH 9), 0.01% gelatin and 0.1% Triton X-100), 1.75 mM MgCl₂, 20 µl Q solution (2X final), 400 µM each dNTP, 1.8 µM each primer and 4%(by vol.) DMSO.

Gene	Primers	2x buffer	annealing	program	size bp
nrITS	ITS-42F/ ITSb+57R	K	50°C	(94-50x30)	1270-1272
petN	petN5F/psbM111R	E	50°C	(94-50x30)	839-845

Primers (5'-3'):

ITS: ITSA = GGA AGG AGA AGT CGT AAC AAG G;

ITSB = CTT TTC CTC CGC TTA TTG ATA TG.

ITSA and IBSB primers from Blattner (1999).

additional ITS primers (based on *Juniperus* sequences):

ITSA-42F = GAT TGA ATG ATC CGG TGA AGT

ITSB+57R = ATT TTC ATG CTG GGC TCT

petN - psbM:

petN5F = AAC GAA GCG AAA ATC AAT CA

psbM111R = AAA GAG AGG GAT TCG TAT GGA

petN and psbM primers were based on conserved sequences from *Juniperus* species.

The following PCR conditions were used: MJ Research Programmable Thermal Cycler, 30 cycles, 94°C (1 min.), 50°C or 57°C (2 min.), 72°C (2 min.), with a final step of 72°C (5 min.). The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit. The gel purified DNA band with the appropriate primer was sent to McLab Inc. for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments were made using MAFFT (<http://align.bmr.kyushu-u.ac.jp/mafft/>).

SNPs analyses

Aligned data sets (nrDNA and trnC-trnD) were analyzed by CLEANDNA (Fortran, R. P. Adams) to remove invariant data. Mutational differences were computed by comparing all SNPs, divided by the number of comparisons over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). A minimum spanning network was constructed by selecting the nearest neighbor for each taxon from the pair-wise similarity matrix, then connecting those nearest neighbors as nodes in the network (Adams et al., 2003).

RESULTS AND DISCUSSION

Analyses of the nrDNA sequences revealed 26 mutational events that included a 2-bp indel (CA) that was present in the three *J.*

martinezii individuals and absent on all other taxa. In addition, one of the *J. flaccida* individuals (6893) contained an insertion (A) that was absent in all other samples. Thirteen of the mutational events were single events and 13 were multiple occurring with fidelity within populations. A minimum spanning network was constructed based on 13 SNPs (including one indel) and is shown in figure 2 (left). The Topia shrubs had no SNPs different from *J. durangensis*. Overall, these taxa appear to

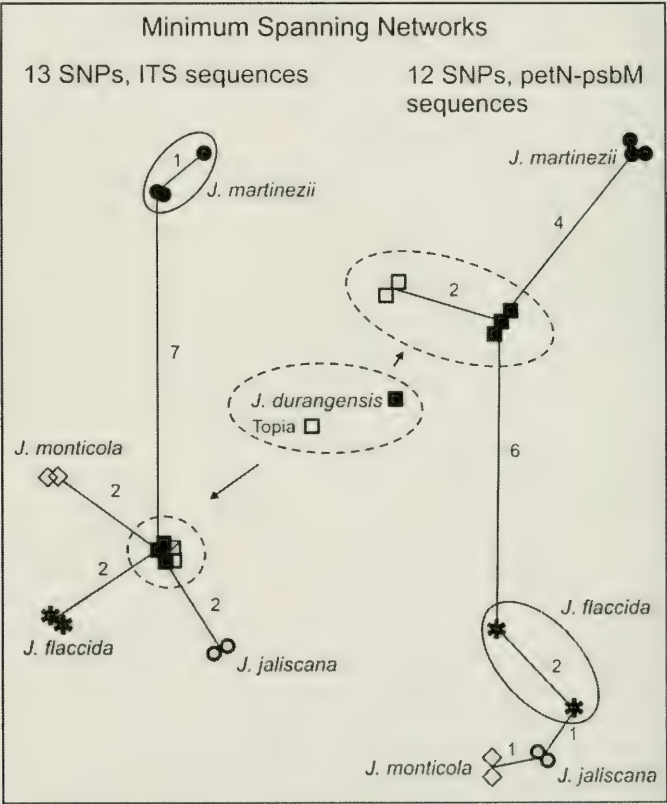


Figure 2. Minimum spanning network based on SNPs from nrDNA (left) and petN-spacer-psbM (right). The number of SNPs are next to the links.

be very closely related, with only *J. martinezii* having appreciable SNPs differences.

Analyses of a petN-spacer-psbM (from cpDNA) revealed 14 mutational events, with 6 of these being indels. Two events occurred in single individuals. Twelve SNPs (including 5 indels) were used to construct a minimum spanning network (Fig. 2, right). The Topia plants had 2 indels (an A at 401 and a deletion at 666) not found in *J. durangensis* (or other taxa). *Juniperus martinezii* is separated by 4 SNPs (Fig. 2, right) and *J. flaccida*-*J. jalisciana*-*J. monticola* are separated from *J. durangensis* by 6 or more SNPs.

Combining the nrDNA and petN-s-psbM data resulted in the minimum spanning network shown in figure 3. Notice that species are

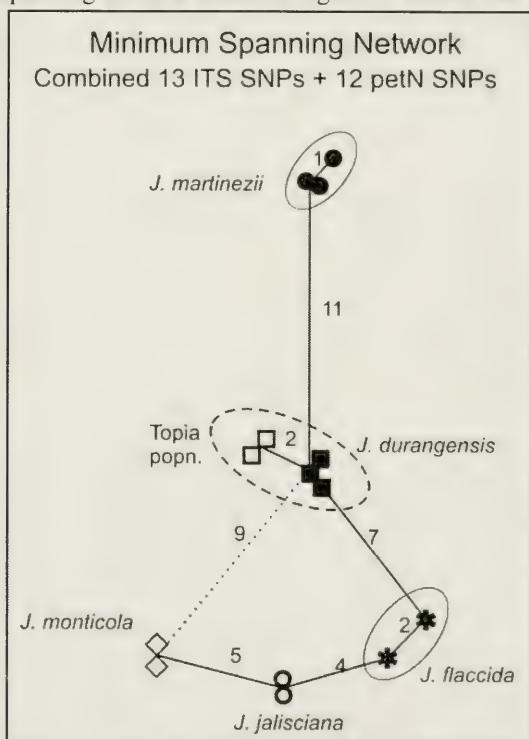


Figure 3. Minimum spanning network based on combined SNPs from nrDNA and petN-s-psbM sequencing. The dotted line is the second shortest link for *J. monticola* (9 SNPs).

separated by from 4 to 11 SNPs. The *Topia* plants are quite near typical *J. durangensis* in these two nucleotide sequences. However, it is clear that conclusions based on a single sequence might be misleading (cf. Fig. 2, left vs. right). Additional collections and analyses of the leaf essential oils of the *Topia* plants, as well as sequencing additional genes, should shed light on the scope of differentiation of this population and its affinities to other junipers.

ACKNOWLEDGEMENTS

Thanks to Andrea Schwarzbach, Socorro González and Billie Turner for manuscript reviews. Thanks to Tonya Yanke for lab assistance and to Socorro González for samples of the *Topia* juniper. This research was supported in part with funds from Baylor University.

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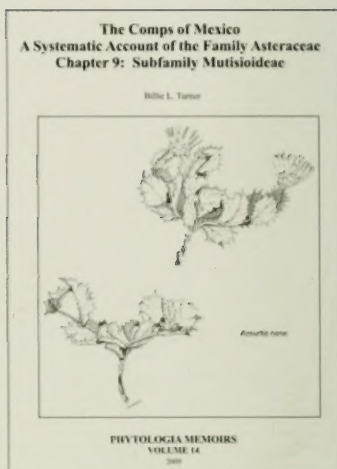
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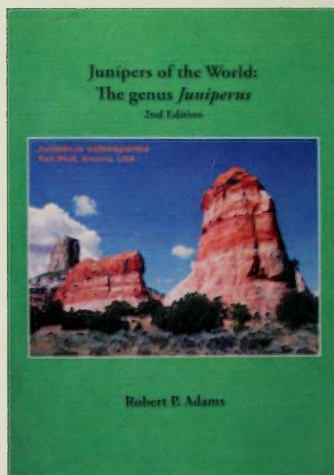


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